

AN ABSTRACT OF THE THESIS OF

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Title: POPULATION DYNAMICS, EXTRACTION, AND RESPONSE  
TO NEMATOCIDE OF THREE PLANT PARASITIC NEMATODES ON  
PEPPERMINT (MENTHA PIPERITA L.)

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The efficiencies of wet sieving/sucrose centrifugation (WS/SC) recovery of Pratylenchus penetrans (59 %), Paratylenchus sp., (80 %), and Criconemella xenoplax (66 %) were established. Baermann funnels (BF) recovered significantly more P. penetrans ( $p = 0.01$ ) and significantly less ( $p = 0.01$ ) C. xenoplax than did WS/SC. While densities of P. penetrans in stored soil remained constant over the three days following field sample collection, Paratylenchus sp. and C. xenoplax densities increased significantly on the second day and decreased to their original level on the third day. During mist chamber extraction, P. penetrans continued to emerge from peppermint root tissue for 38 days, but 90 % of the total was recovered after 10 days.

The standard core, consisting of 500 g dry soil plus the roots and rhizomes in that soil, was developed to

express endoparasitic and ectoparasitic nematode densities in peppermint field soil, roots, and rhizomes.

Enumerating nematode densities within the different plant-soil components of a particular volume of soil more closely describes the total nematode population pressure on the plant growing in that volume of soil. Therefore, endoparasitic nematode population levels were expressed as numbers in standard core soil, roots, rhizomes, or total core (soil, root, and rhizome populations combined).

P. penetrans populations in peppermint fields peaked in early May, decreased through the summer, peaked again in August, and decreased through the fall to a low winter level. Peaks in the P. penetrans population followed peppermint root weight peaks by 3 to 6 wks. Paratylenchus sp. populations remained at relatively low levels throughout the year except for a pronounced peak in August, which followed the root weight peak by 3 to 6 wks. The C. xenoplax population also peaked 3 to 6 wks after the August root weight peak but fluctuated markedly throughout the remainder of the year. From 70 to 90 % of the total P. penetrans population was in roots in early May, decreased to 40 to 50 % by late June and 20 to 40 % in August. Up to 20 % of the population was in rhizomes on some dates, but the rhizome percentage was usually less than 10. Fewer P. penetrans were recovered from rhizomes

during the harsh winter of 1988-89 than during the mild winter of 1989-90.

Analysis of point samples (pretreatment, posttreatment, and harvest samples) and area under nematode population curves (AUNPC) were used to compare nematode populations in oxamyl-treated (1.1 kg a.i./ha) and nontreated plots in two peppermint fields through the two growing seasons. Point sample analyses detected significant decreases in treated soil, root, and total standard core P. penetrans populations compared to nontreated populations in several pretreatment and harvest sample dates and in two rhizome harvest sample dates. No treatment differences were observed in Paratylenchus sp. or C. xenoplax populations using this analysis. AUNPC analysis detected significant decreases in several treated root and rhizome P. penetrans populations compared to nontreated populations and in total core populations in field 1 during one growing season and in field 2 during two growing seasons. Significant decreases in C. xenoplax populations were observed in one field during one growing season.

Peppermint hay weight was significantly greater in treated than in nontreated plots in one of three fields in 1988 and in one of three fields during 1989. Oil in ml/kg fresh hay weight was significantly lower in treated than in nontreated plots in one of three fields during 1989.

No treatment differences were detected in milliliters of oil distilled from 2m<sup>2</sup> field area.

Peppermint oil production is the final measure of a treatment from a mint grower's perspective. Because oxamyl had no effect on mint oil production, AUNPC appears to be a better measure of parasitic nematode pressure on peppermint, since this method of analysis detected fewer significant differences between nematode populations between treated and non-treated plots.



POPULATION DYNAMICS,  
EXTRACTION,  
AND RESPONSE TO NEMATOCIDE  
OF THREE PLANT PARASITIC NEMATODES  
ON PEPPERMINT (MENTHA PIPERITA L.)

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## DEDICATION

To Cinnamon,  
Brillo,  
and the late Noodle

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INTRODUCTION

Peppermint (Mentha piperita L.), a member of the Labiatae, is generally thought to be a natural hybrid, probably originating in England, collected from the wild and cultivated due to its pleasant taste and aroma. The flowers, although morphologically typical of the mint family, are sterile, and propagation is by means of rhizomes. The essential oil, which is concentrated in leaf glands, was distilled as early as A.D. 410 by the ancient Egyptians. The oil has been employed in various capacities since that time and is currently used as flavoring in medicines, confections, toothpastes, and chewing gum.

Peppermint was first grown commercially in Mitcham, Surrey, England around 1750. After reaching its climax 50 years later, the English industry declined, probably due to American competition. Commercial culture in the United States began in Ashfield, Massachusetts in 1812. The New York peppermint industry was initiated in 1816, expanded rapidly, and then declined in the early 1900s due either

to competition with the new peppermint industry in Michigan or to insects, disease, and depleted soil fertility. The industry reached its greatest development in the muck soils of Michigan and neighboring Indiana from 1935 to 1942. Contamination of these muck soils with Verticillium dahliae Kleb., the cause of Verticillium wilt, and competition with the new Pacific Northwest mint industry contributed to the decline of Michigan and Indiana mint production. Peppermint is currently grown commercially in northern latitudes of both the midwestern and far western United States. In 1989, peppermint acreage in the United States exceeded 45,000 hectares, 18,600 of which were in Oregon. Nearly half of Oregon's peppermint acreage is in the Willamette Valley.

Diseases and pests of peppermint in Oregon include Verticillium wilt, mint rust, mint root borer, mint flea beetle, two-spotted spider mite; bindweed, groundsel, and other weeds; and plant-parasitic nematodes. Horner and Jensen (1954) reported Paratylenchus macrophallus, Aphelenchoides parientinus, Meloidogyne hapla, and Longidorus sp. to be associated with peppermint plantings. As awareness of plant-parasitic nematodes increased, much of the vague stand damage traditionally attributed to soil infertility or to unknown causes was associated with nematodes.

The dominant plant-parasitic nematode collected from declining peppermint fields in western Oregon has been Pratylenchus penetrans. Reductions of 46% in peppermint foliage and 86% in roots have been observed in laboratory cultures infested with P. penetrans (Bergeson and Green, 1979), and P. penetrans densities in August root samples were negatively correlated with yield (Pinkerton, 1983). Plants damaged by this species generally have weak, shallow root systems and form patchy stands with extensive bare areas in which weeds may become established. Measurements of living, water-mounted females from populations recovered in the present study are within the range for P. penetrans (Handoo and Golden, 1989).

Peppermint yield losses in the Willamette Valley have also been attributed to damage by Paratylenchus sp. (Ingham and Morris, unpublished data). The Paratylenchus dealt with in the present paper is the same species and in some cases is from the same field. Measurements and characteristics of the present species resemble those of P. microdorus, P. hamatus, and P. dianthus, as well as those of the originally described P. macrophallus, but enough differences remain to refrain from species assignment at this time (Raski, 1975a; Raski, 1975b, Raski, 1962; Tarjan, 1960; Goodey, 1934. Determined from 8 living, water-mounted females,  $L = 392.8 \text{ u}$  (355.6 to

403.3), stylet length = 27.3 u (27.1 to 27.7), v = 76.2 (68.8 to 79.1), a = 22.0 (15.8 to 26.2), and b = 4.3 (3.8 to 5.9).

Criconemella xenoplax has been noted increasingly in recent years in peppermint soil samples from western Oregon. No data on its effect on peppermint are available. Although this species is shorter in body and stylet length than the originally described C. xenoplax, the specific limits were extended by collections from New Mexico forest soils (Raski and Riffle, 1967; Raski, 1952; Siddiqi and Goodey, 1965; Raski and Golden, 1965; Tarjan, 1966). Determined from 5 living, water-mounted females, L = 418.3 u (361.8 to 478.4), stylet length = 63.8 u (55.3 to 71.2), a = 12.3 (11.8 to 15.0), v = 90.7 (89.7 to 90.2), and b = 3.6 (3.2 to 4.7).

Oxamyl, a systemic carbamate nematicide, is applied to peppermint fields in the spring, usually late April, to suppress plant-parasitic nematode populations. This product has successfully rejuvenated stands severely damaged by P. penetrans and L. elongatus. Early spring oxamyl applications produced significant yield responses (Pinkerton, 1983).

The purpose of the present study is to provide basic data on the population levels of P. penetrans, Paratylenchus sp., and C. xenoplax on peppermint in the

Willamette Valley and to assess the effect of oxamyl on populations of these three species. In order to provide an evaluation of population measurement, the efficiency of extraction of these three species from soil (and of P. penetrans from roots and rhizomes) is examined.

Population dynamics of each of the three species are described for the two year study period. The effect of oxamyl on the population levels of each species is analyzed, and hay and oil yields from treated and non-treated plots are analyzed. It is hoped that the information gained from this study will help to address issues such as the most efficient extraction of target species and the timing of control or sampling practices as well as to provide a basis for directing further inquiries.

## LITERATURE REVIEW

### PRATYLENCHUS PENETRANS AND OTHER PRATYLENCHUS SPECIES.

The genus Pratylenchus is comprised of vermiform nematodes from 340 to 800 microns long and with a length to width ratio of 15 to 35 (Dropkin, 1970). Members of the genus are characterized by a flattened head, strong cephalic framework, and a short, thick stylet with prominent basal knobs, ventral overlap of the intestine by the esophageal glands, excretory pore near the level of the esophago-intestinal junction, vulva at about 70 to 80% of the body length, short postvulval sac on the ovary, fine annulations, and usually four lateral lines. P. penetrans (Filipjev and Schuurmans Stekhoven, 1941) is characterized by a lip region composed of three annules, a smooth tail terminus, a round or somewhat square and subspherical spermatheca, a short, undifferentiated posterior uterine branch, a long, slender body, average a of  $>25$ , a lip region low and slightly set off, oral aperture with six adjoining sensilla, with males common (Handoo and Golden, 1989). P. penetrans with crenate tails, variously shaped stylet knobs, and variation in a, b', and c ratios were observed by Tarte and Mai (1976).

Specimens of P. penetrans from peppermint obtained from Washington resembled the type specimen.

**Life cycle:** Pratylenchus species are migratory endoparasites and usually have a wide host range (Dropkin, 1989). Eggs are laid in clusters in roots or soil. Second stage juveniles (J2) hatch following the first molt in the egg. All stages move between soil and roots (Dropkin, 1989), and all life stages were observed in infected roots (Oyekan et al., 1972). Life cycle duration is greater at low temperatures (15 C) than at high temperatures (30 C) (Maniyya, 1971). The incubation period of eggs ranged from 25 days at 15 C to 9 or 10 days at 30 C, and the number of progeny produced was apparently not temperature dependent. Females laid an average of 1.1 eggs each day for about 35 days at 24 C, and the maximum number of eggs laid by a female was 68. DiEdwardo (1960) found that the P. penetrans egg shell was rigid until the last 48 to 56 hours of development, during which frequent, vigorous movement alternated with brief periods of quiescence. During these last developmental hours, the stylet was thrust repeatedly against the membrane at one end of the egg, and the egg shell became thin and plastic. The J2 became free within seconds of breaking the end of the shell. Reproduction did not occur without males, and cytological examination indicated that cross-fertilization

occurred (Thistlethwaye, 1970; Maniyya, 1971). Eggs of virgin females failed to undergo cleavage. Males reared in isolation produced sperm. Females' chromosome number is  $2n = 12$ . Results of concurrent studies on potato and corn indicate that host crop has a significant influence on the within-generation dynamics of P. penetrans (MacGuidwin, 1987).

**Host Range:** Jensen (1959) found that 33 graminaceous and leguminous crops tested were hosts for P. penetrans, which was widely distributed in Oregon on various bulb and nursery crops. Apple and peach are both hosts, but apple is considered less sensitive, since it can sustain P. penetrans in the cortex for over one month without serious injury (Potter et al., 1960; Parker and Mai, 1950). Townshend and Davidson (1960) investigated strawberry weeds as hosts and found many host species, 63% of which were in the Compositae and Cruciferae. Perennial weed species with soft-textured roots contained larger P. penetrans populations and had more extensive root necrosis than species with hard-textured roots. Tomato, cabbage, lettuce, cauliflower, eggplant, and potato are P. penetrans hosts, and this species can overwinter in celery root debris (Townshend, 1962a). Wong and Ferris (1968) found potato and peppermint better hosts of P. penetrans than onion. P. penetrans is pathogenic to the annuals



zinnia and garden balsam (Heald, 1963). Sudangrass, sudex, and oats were the poorest P. penetrans hosts and thus the most highly recommended cover crops when compared with rye, wheat, buckwheat, and Japanese millet (Dunn and Mai, 1973). In another cover crop study, Sudangrass and Buckwheat were found to be the best hosts, whereas perennial ryegrass and creeping red fescue were the poorest hosts and thus recommended as cover crops (Marks and Townshend, 1973). P. penetrans reproduced well on birdsfoot trefoil, red clover, and alfalfa, in decreasing order (Willis et al., 1982). Timothy supported an increase as well, but yield was not affected. Population densities of P. penetrans on potato, oat, and corn increased more than did those on rye, wheat, and sorgho-sudangrass in pot studies (Florini and Loria, 1990).

**Behavior, Feeding, and Movement.** Pratylenchus penetrans movement was related to moisture tension and not to moisture content (Townshend and Weber, 1969). The maximum distance that nematodes moved in seven days was 2 cm when each soil was of low bulk density and when moisture tension was optimal. DiEdwardo (1960) observed adults moving as much as 2 mm per minute. Adults and J4 moved significantly further in sand than in loam. Lavelee and Rhode (1962) found P. penetrans movement in agar significantly greater in the direction of host seedlings

than in other directions. Seedlings were not attractive when placed more than 12.5 mm from the inoculation site, and excised root tips were not attractive to nematodes.

In cabbage roots, P. penetrans probed endodermal cells two weeks after inoculation and penetrated the stele four weeks later, causing localized mechanical destruction (Acedo and Rohde, 1971). After penetrating the epidermis intercellularly and aligning longitudinally in the hypodermis, P. penetrans penetrated transverse walls of successive cells, and entered the cortex to feed and reproduce (Potter, et al., 1960; Townshend, 1963b). Movement in heavily infected roots appeared unrestricted and apparently caused mechanical damage by forming galleries in the cortex (DiEdwardo, 1960; Townshend, 1963a).

Conditions most suitable for maximum movement of Pratylenchus sp. in soil and penetration of roots were the least suitable for maximum survival (Townshend and Webber, 1971). Survival may be highest at low moisture and temperature just above freezing, but these conditions immobilized the nematodes (Townshend, 1972). Such nematodes would likely have a lower metabolic rate and should deplete their food reserves more slowly, thus enhancing their survival over long periods without a host.

**Feeding Site Selection.** Freckman and Chapman (1972) found that the preferred site of penetration was 3 to 13 mm posterior to the root tip, and Townshend (1978) found that the dense root hair zone was preferred. P. penetrans invaded there first but then along the entire root from tip to radical-hypocotyl juncture (Freckman and Chapman, 1972). Penetration was relatively rapid, and from 75 to 90% efficient. Nematodes did not readily penetrate new root tissue distal to previously invaded sites, and there was no consistent relationship between sites of penetration and sites of branch root initials. Kurppa and Vrain (1985) found that most P. penetrans migrated to the root hair zone within three hours. Some nematodes penetrated through root hairs but did not feed on them.

After exploration of potential feeding sites, P. penetrans selected an epidermal cell by rubbing it with lips and stylet (Kurppa and Vrain, 1985). The stylet was thrust into cells and withdrawn up to 140 times per minute (DiEdwardo, 1960). Nematodes bored several holes in the cell wall and then forced their way through. This was followed by the injection of saliva, which appeared in the cell as opaque droplets (Kurppa and Vrain, 1985). If the function of saliva is to lower the viscosity of cell contents, the slow pumping of the metacarpus may indicate monitoring of viscosity. Ingestion follows salivation.

**Root Penetration.** Penetration of roots by P. penetrans adults was greater than penetration by juveniles in all soils (Townshend, 1972). P. penetrans moved 7 mm in 4 hours towards peach roots (Mountain and Patrick, 1959). Within 90 minutes of arrival, a discolored area appeared on the root. P. penetrans had not completely entered pea roots six hours after inoculation, although some had begun (Oyekan et al., 1972). Most penetrated roots by 12 hours after inoculation, and by 18 to 24 hours, most were in the mid-cortex. Maximum penetration by females occurred between 10 and 30 C and males and J3 at 20 C (Townshend, 1978).

**Internal vs. External Feeding.** Rebois and Huettel (1986) observed extensive external feeding by P. allenii in lab cultures, and they concluded that ectoparasitism is common in this species and not a byproduct of support medium inhibition. In excised root tissue cultures, P. agilis was capable of ectoparasitic as well as endoparasitic feeding. Female and J3 P. penetrans entered roots 122% and 83%, respectively, more often than did males (Olthof, 1982). P. penetrans penetrated deeper into Stellaria media roots than did P. crenatus, but P. penetrans sometimes had difficulty penetrating the cuticle, especially that of older roots (Klinkenberg,

1973). Both Pratylenchus species entered most roots on which they fed except the very thin ones.

**Disease Caused by P. penetrans: Lesion Appearance.**

Alfalfa root lesions caused by P. penetrans initially appeared as water-soaked areas at the root surface and later became yellow and elliptical as nematodes entered the cortex (Townshend, 1978). Dark brown cells later appeared in the center as nematodes fed. DiEdwardo (1960) and Townshend (1963a) report gallery formation in the cortex. Discreet lesions and large brown areas in carrot roots appeared 17 days after inoculation (Rohde, 1971). Apple root cell contents became disorganized when invaded by P. penetrans, and light brown and yellow spots appeared (Potter, et al., 1960). The roots reacted to P. penetrans within three hours with a yellowing of small groups of cells around nematodes in the epidermal region. Dark streaks in the stele region appeared after three or four weeks.

**Endodermal Penetration.** Potter et al. (1960), Townshend (1963a), and Oyekan et al. (1972) found that endodermal cells of apple, celery, and pea, respectively, were a barrier to P. penetrans movement. Although strawberry endodermal cells were not invaded, they became discolored first and most intensely after invasion, while the cortex and epidermis discolored later and less

intensely (Townshend, 1963b; Oyekan et al., 1972). P. penetrans entered and fed only on cortical cells in potato roots, rhizomes, and tubers (Dickerson et al., 1964).

Endodermal penetration has been documented in some cases. Acedo and Rohde (1971) observed stele penetration in cabbage, carrot, and tomato roots. In carrot and tomato, the endodermis was temporarily resistant to penetration, but P. penetrans was found in the stele one month after inoculation. Although strawberry endodermal cells were not invaded, they became discolored first and most severely, while the cortex and epidermis discolored later (Townshend, 1963b). In cabbage roots, P. penetrans probed endodermal cells two weeks after inoculation and penetrated the stele four weeks later, causing localized mechanical destruction (Acedo and Rohde, 1971).

**Effect of P. penetrans on root cells.** Nematodes and eggs in various stages of development were seen in amber colored lesions but not in dark brown lesions, due to opaque cell walls (Townshend, 1962b). Eggs were observed in the cortex five days after inoculation (Townshend, 1963b). Celery root steles could be nearly ensheathed by P. penetrans when many nematodes entered the root in a small area (Townshend, 1963a).

P. penetrans caused necrosis and breakdown of cabbage root cortical parenchyma, possibly due to accumulation of

phenolic compounds and activation of associated oxidative enzymes (Acedo and Rohde, 1971). Necrosis within P. penetrans-infected peach root tissue was rapid and appeared to advance before the invading nematode, with discolored areas 2 cm in length present 96 hours after invasion (Patrick and Mountain, 1959). Lesions were formed mainly by the production of phytotoxic substances from hydrolysis of cynaophoric beta-glucoside amygdalin. P. penetrans hydrolized this substance in vitro. Discolored cells showed high concentrations of phenolic substances, while healthy cortical parenchyma did not. Celery root epidermal, cortical, and endodermal cellular contents were disorganized and discolored, accumulating at the cell wall as well as in intercellular spaces. P. penetrans damaged pepper (Capsicum frutescens) by mechanically destroying parenchyma cells in the root cortex (Shaffiee and Jenkins, 1963).

**Host response to Pratylenchus spp.** Penetration and feeding by Pratylenchus spp. may stimulate injured cells to accumulate phenolic compounds (Klinkenberg, 1963; Acedo and Rohde, 1971), which may be converted to phytotoxic substances by enzymes normally present in celery roots (Townshend, 1963a). Phenols accumulated in carrot and tomato endodermis far in advance of nematode feeding (Acedo and Rohde, 1971). More potassium, nitrogen,

phosphorus, and sodium accumulated in significant quantities in roots infected by P. penetrans than in non-infested plots (Shaffiee and Jenkins, 1963). Potassium also accumulated in leaves of infected plants.

Chlorogenic acid, a major phenol of tomato roots, attracted P. penetrans, but when this compound was oxidized to a yellowish brown compound, it was repellent (Chang and Rohde, 1969). Peroxidase activity increased five-fold near lesions in cabbage roots (Acedo and Rohde, 1971). Phaseolin, a bean phytoalexin, was isolated from aseptically grown Phaseolus vulgaris seedlings infected with axenized P. penetrans (Abawi, et al., 1971).

P. scribneri stimulated an apparent hypersensitive reaction in lima bean, and only minimal reproduction and localized damage occurred (Thomason et al., 1976). In contrast, a lack of cellular necrosis in snap bean and prolific nematode reproduction suggested that P. scribneri could move freely through epidermal and cortical cells. Thus snap bean appeared to possess little or no mechanical or chemical resistance.

Occasional hypertrophy of celery cortex resulted in slightly swollen roots (Townshend, 1963a). Pectic-material plugs were associated with cabbage root cortical injury, although this was probably a host response to injury rather than a form of resistance (Acedo and Rohde,



1971). Necrotic host tissue had a repellent effect on P. penetrans (Chang and Rohde, 1969).

**Effect of Physical Environmental Factors on Pratylenchus spp. populations: Temperature: P. penetrans**

mortality in soil in lab studies at both -12 C and -8 C was about 93% after four hours exposure (Kimpinski, 1985). The duration of exposure needed to kill 50% at -4 C, 0 C, and 4 C was 3.4, 124, and 742 days, respectively. The P. penetrans life cycle was completed in 86 days at 15 C, in 42 - 44 days at 20 C, in 35 days at 24 C, and in 30 - 31 days at 30 C (Maniya, 1971). At 33 C, development was inhibited. The optimum temperature for corn root penetration was 20 C for P. penetrans and 30 C for P. minyus (Townshend, 1972).

The optimum temperature for population development of P. allenii was 30 C and for P. scribneri, 30 - 34 C (Acosta, 1982). Stress on the soybean plants at 34 C reduced their tolerance to the thermophilic P. scribneri, reducing plant growth. Acosta and Malek (1979) found population increase on Clark 63 soybean most rapid at 30 C for P. allenii, P. brachyurus, P. coffeae, P. neglectus, P. scribneri, and P. zeae and at 25 C for P. penetrans and P. vulnus, the latter two being the only species reproducing at 15 C. P. penetrans populations increased from 20 to 30 C but did not reproduce at 35 C. The optimum temperature

for reproduction of P. vulnus on carrot in lab cultures was 26 C, with reproduction decreasing sharply at higher or lower temperatures. Motility temperature optimum was 19 C, although motility was not significantly reduced over the entire temperature range. The optimum temperature for P. hexincisus increase on corn in four soils was 30 C (Zirakparvar et al., 1980). P. penetrans reproduction was higher at 30 C than at 22.5 C and 15 C (Patterson and Bergeson, 1967). Numbers of P. penetrans in soil at depths of 0 to 15 cm decreased 35 to 59% from December to April when field soil temperatures ranged from -1.1 to -0.8 C (Kimpinski, 1985). Nematode numbers in this study increased 39% during one winter when soil temperatures ranged from 0.1 to 0.4 C.

**Effect of Temperature on the Effect of Pratylenchus spp. on Plant Growth and Yield.** Final soil and root populations of 20,300 and 52,290 P. alleni per soybean pot caused no detectible effect on growth at 34 C (Acosta, 1982). Both similar and lower populations of P. scribneri (55,120 and 16,410) at this temperature inhibited growth. Both species reproduced at all temperatures between 22 and 34 C, but population increase for both was least at 22 C. At temperatures from 7 to 13 C, less than 100 P. penetrans per gram root caused significant reduction in onion seedling root weights by the fifth week after germination,

but more than 400/g were required to produce comparable injury at 16 to 25 C (Ferris, 1967). P. penetrans per gram dry alfalfa root increased more rapidly at 27 C than at 10 or 18.5 C, but increase in soil was more rapid at 10 C than at the other two temperatures (Kimpinski and Willis, 1978). Disease caused by P. vulnus was more severe at 29.5 C than at 18.3 C or 23.9 C (Sher and Bell, 1965).

**pH.** P. penetrans increase was significantly greater at pH 5.2 and 6.4 than at 4.4 and 7.3 on alfalfa in fine sandy loam (Willis, 1972). Nematode infestation significantly decreased forage yields at pH 5.2 and 6.4 but not at 4.4 and 7.3. Pratylenchus penetrans numbers increased significantly as pH increased (Kimpinski and Willis, 1978). Mean populations were 35, 587, and 594 at pH 4.9, 6.3, and 7.3 per pot, respectively. The optimum pH for movement of P. penetrans was 6.0 (Kimpinski and Willis, 1981).

**Soil Moisture.** The rate of population increase of Pratylenchus penetrans was greatest at moderate soil moisture tensions (pF 2 - 3) and least at very low or very high tensions (Kable and Mai, 1971). The number of nematodes surviving in soil generally decreased with increasing moisture and temperature above freezing. Nematodes remained viable at tensions as high as pF 5.0,

but death was rapid at pF 5.06. Population increase was significantly restricted by pF 3.0 (Willis and Thompson, 1969). Under a range of moisture tensions from 0 to 1000 cm water, the root penetration peak was between 10 and 100 cm (Townshend, 1972). During high rainfall, P. brachyurus suppressed soybean growth and yield (Koenning et al., 1985). Root penetration may be favored by moist soil, but high soil moisture may suppress root growth by decreasing oxygen. The injury mechanism may involve interactive effects of root growth suppression by excessive soil moisture and nematode feeding.

**Soil Texture.** P. penetrans damage to fruit trees in New York was greatest in light textured soil, present but less damaging in medium textured soil, and apparently nonexistent in heavy soils (Parker and Mai, 1956). Disease of roses caused by Pratylenchus vulnus was more severe in light sandy soil than in silty clay loam (Sher and Bell, 1965). Lower bulk densities favored root penetration by P. penetrans and P. minyus in three soils (Townshend, 1972). Nematode penetration of roots in sandy loam was greater than in silt loam and loam. Survival of these two species in columns of loamy sand and silt loam without host plants declined with temperature as it increased above 2 C (Townshend, 1973). Neither species survived sub-zero temperatures. More P. penetrans in this

study survived in loamy sand than in silt loam. P. hexincisus populations on corn were significantly greater in coarse sand than in silt loam or silty clay loam at 30 C (Zirakparvar et al., 1980).

The results of Kable and Mai (1985b) suggested that overwintering survival of Pratylenchus penetrans at depths of 30 cm or greater is less in clay soil than in sandy soil. This might contribute to the lower population of nematodes in clay soils, but before this questions is solved, the relative importance of soil, senescent roots, and living perennial roots as overwintering medium must be studied. No great difference was detected in survival within roots in sandy loam versus clay loam soils.

Pinkerton (1983) found soil texture, P. penetrans peppermint root population, and peppermint hay yield to be significantly correlated. Yield was negatively correlated with per cent sand and positively correlated with percent silt. P. penetrans soil and root populations were positively correlated with sand and negatively correlated with silt.

**Soil Moisture and Soil Texture Interaction.** Soil moisture and soil texture may interact to affect nematode populations. Townshend and Weber (1971) suggested that the relatively large grain size of a sandy soil provided pore sizes and moisture characteristics more suitable for

P. penetrans movement than conditions in loam or silt loam. Higher silt and clay contents of soils required greater moisture tensions for satisfactory P. penetrans population growth (Kable and Mai, 1968a). At pF 3.7 to 4.2, survival was much greater in clay than in sandy soils. Some P. penetrans in sandy soil entered alfalfa roots under either saturated (pF 0) or dry conditions (pF 3), but entry was greatest under moist conditions (pF 1.8 to 2.5). These authors suggested that the widespread occurrence of high Pratylenchus spp. in sandy soils could be explained in terms of the interaction of soil moisture with soil type.

Conditions most suitable for maximum movement of Pratylenchus sp. in soil and penetration of roots were the least suitable for maximum survival (Townshend and Webber, 1971). Survival may be highest at low moisture and temperature just above freezing, but these conditions immobilized the nematodes (Townshend, 1972). Such nematodes would likely have a lower metabolic rate and should deplete their food reserves more slowly, thus enhancing their survival over long periods without a host.

**Host Plant Influence on Pratylenchus spp.**  
**populations.** The nitrogen metabolism of peppermint (Mentha piperita) in pot cultures was altered when the

calcium/potassium ratio was changed from <1 to >3 (McDonald, 1969). Plants receiving solution with 1:3 Ca to K ratios contained more P. penetrans than those receiving 3:1 Ca to K. This was probably due to altered nitrogen metabolism in the plant. P. penetrans reproduction was favored in roots of hosts fertilized with nutrient combinations low in nitrogen (Patterson and Bergeson, 1967). Migration of this species from roots to soil was increased when the host received no nitrogen or phosphorous in fertilizer or by short photoperiods and high temperatures.

P. hexincisus numbers in fibrous roots of corn were greater than those in coarse roots (Zirakparvar, 1979). Jaffee (1980) found a negative correlation between root biomass and number of P. penetrans per gram alfalfa root tissue. Penetration by all migratory life stages was inversely proportional to tissue age and may be related to greater resistance in older plants (Olthof, 1982).

#### **Effect of P. penetrans on Host Growth and Yield:**

**Yield Reduction and Stunting.** Growth of Trifolium pretense and Medicago sativa were significantly reduced by P. penetrans (Chapman, 1959). Symptoms of P. penetrans infestation on potatoes were circular areas 30 to 150 feet in diameter, reduced vigor, yellowing, cessation of growth in the latter part of the season, reduced root systems,

and lower yields (Dickerson et al., 1964). Top and root growth of Lotus corniculatus was significantly reduced by P. penetrans (Willis and Thompson, 1969).

P. penetrans was closely associated with irregular areas of stunted, orange-rooted celery (Townshend, 1962a). Large numbers of nematodes were required to produce stunting in celery in growth chamber experiments. P. penetrans was also correlated with a specific type of root lesion and stunting of strawberry, and again, large numbers were required to produce stunting under controlled conditions (Townshend, 1962b). Celery seedlings were stunted one week after P. penetrans inoculation, but later, the growth of inoculated and non-inoculated plants exhibited similar growth (Townshend, 1963a).

Oostenbrink (1961) observed a significant linear relationship between the log of initial population density in roots and the growth deficit of crops. This varied between crops, soils, and years, and with instrument standardization. Acosta (1982) found the degree of Pratylenchus spp. damage to be influenced by the initial nematode density and by temperature. P. penetrans population levels in roots were negatively correlated with peppermint yield, while densities of ectoparasitic nematodes and P. penetrans soil populations did not improve regression models (Pinkerton, 1983). P. penetrans



harvest and preseason root populations were negatively correlated with yield. Yield reductions of 63% and 38%, respectively, accompanied each 10-fold increase of mean P. penetrans root populations above the tolerance level (population on the regression line where yield equals 100%). Because P. penetrans feeding in the root cortex damage root systems, root populations should be proportional to damage. Soil populations, however, showed no correlation with yield.

**Yield Reductions in Varieties of Differing Resistance.** Bergeson and Green (1979) found three peppermint cultivars -Black Mitcham, Murray's Mitcham, and Todd's Mitcham - equally susceptible to P. penetrans infestation. Populations of 5000 per one-cutting pot did not significantly affect foliage weights from either of two cuttings of the three cultivars compared with non-inoculated plants. However, root weights of Black Mitcham and Todd's Mitcham were significantly reduced by 48% and 41%, respectively. In an additional experiment, a significant reduction in dry weight of foliage from the first cutting of Todd Mitcham and Murray's Mitcham (40% and 39%, respectively) was observed compared to non-inoculated plants. Reduction at the second cutting was significant for all three cultivars. Reductions of 70 to 86% in root weights were significant compared to control

plants. In near sterile conditions, P. penetrans reduced peppermint foliage growth up to 34% and root growth up to 66% (Bergeson, 1963).

The effect of the preceeding crop upon P. penetrans and P. minyus root disease of tobacco was found to be related to Pratylenchus sp. populations (Mountain, 1954).

**Distribution of the Population in the Plant-Soil System.** DiEdwardo (1961) observed that old strawberry roots were heavily leisoned by P. penetrans, while young roots were relatively lesion-free. From separate extractions of young and old roots, he found that populations in young roots averaged 250 per gram and those in "regular" roots averaged 3200 per gram.

Average P. penetrans male, female, and juvenile precentages in soil and roots were 12, 45, and 43%, respectively (Patterson and Bergeson, 1967). Deviations from these percentages were most noticeable at 15 C, where males and females were more numerous in soil and juveniles more numerous in roots than the above percentages. Increases in densities of P. penetrans in potato roots were associated with a decline in soil populations, and decreases in root population densities were associated with increased soil populations, indicating migration between roots and soil (Bird, 1977).

**Population Dynamics of Pratylenchus spp.** Goheen and Williams (1955) found maximum P. vulnus root populations in cultivated brambles approximately June 1, after which the population declined rapidly and remained low until growth started again in spring. P. vulnus on peach occurred in high numbers throughout the year with a rapid increase during August through December associated with feeder root deterioration (Filegal, 1969).

Barker (1968) found P. zeae populations on various crops highest in October and January through May. Populations of P. zeae on peach were low during January through June and high during September through December but did not correlate with tree vigor (Filegal, 1969).

P. brachyurus soil populations around maize peaked in June, root populations peaked in July (Egunjobi, 1974). In soil around weeds, relatively low populations peaked in October. The majority of P. brachyurus populations from soybean roots and soil was always collected from root fragments (Koenning et al., 1985), and correlations of the root fraction of the population with the total population were always significant. Soil populations correlated poorly with the total population. Eggs of this species were rarely recovered from the soil. Low populations of P. brachyurus on peach were supported all year (Filegal, 1969).

P. scribneri populations on potato increased fourfold or more by mid-season but declined by harvest to levels not significantly different than preplant levels (MacGuidwin, 1987). About 50% to 70% of populations, respectively, were recovered from roots during and after the growing season. Most overwintering mortality occurred before the soil froze, after which all life stages increased but J4 were the most abundant. The same species on both corn and potato roots tended to increase in soil and roots over the growing season (MacGuidwin, 1989). More nematodes were recovered from roots than from soil on most dates, but numbers in most roots decreased in September. Females were the most abundant stage at planting, but the abundance of all stages was similar by harvest. Approximately 50% of P. scribneri in corn and potato overwintered in dead roots. The percentage of the population within roots was 50% at the beginning and end of the growing season and 20% during the growing season.

On strawberries, P. penetrans in soil peaked in June, while the root population peaked in July (DiEdwardo, 1961). The soil population declined from July to September and was lowest in January, but the root population was stable from October through January and increased from March through July. Roots declined in June during fruiting, but after July, root systems were almost

completely renewed. This increase in root growth caused a decrease in the nematode per volume root ratio. After the July peak, the root population decreased rapidly until it began to increase through September.

P. penetrans populations under a rye-tobacco rotation were generally low in summer and high in fall, but seasonal changes were not consistent (Olthof, 1971). Winter mortality in both soil and roots was from 40% to 65%. P. penetrans invaded red clover roots by June 21, three weeks after seeding (Kimpinski, 1975). Populations increased sharply in early August, again in mid-September, and in early November, coincident with production of four generations in roots. A mean soil temperatures of 3.4 C during the latter part of this period indicated that P. penetrans can reproduce at low soil temperatures.

P. penetrans in potato roots increased rapidly for the first three weeks after planting, declined, and increased again in week 5, 6, or 7 (Bird, 1977). Timing and number of further peaks varied among cultivars, and if each peak and following decline represent one generation, there were three to four generations per growing season. Population increases of P. penetrans on various crops in pot studies were not related to root weight or to nematode densities in roots (Florini and Loria, 1990).

Kable and Mai (1968b) found that all motile P. penetrans stages overwintered in either soil or senescent roots. The number of nematodes surviving winter in senescent roots was not significantly affected by soil type or depth. However, the population fraction surviving in soil as opposed to roots in a given soil varied with depth. There was no apparent difference in winter survival between P. penetrans in roots and those in free potato field soil (Dunn, 1972). However, much more of the surviving population in April was recovered from soil than from roots. Substantial numbers of eggs were present in root tissues through the winter, and data suggest that embryonic development and maturation can take place at temperatures as low as -1 C.

Mean P. penetrans root population in western Oregon peppermint peaked in late May and declined in early August (Pinkerton, 1983; Pinkerton and Jensen, 1983). A slight increase until early October was followed by decline during November and December. Soil had two population peaks. The first was in May, two weeks before root populations peaked, and the second peak began in early August and reached a maximum in mid-September. Soil populations peaked before root populations in the fall as nematodes migrated from senescent roots into soil. Both root and soil populations were lowest in late winter.

### PARATYLENCHUS SPECIES

Ranging from 180 to 600 microns, Paratylenchus spp. are the smallest known plant parasitic nematodes. The female's delicate stylet has spheroid basal knobs, the cuticle has fine annulations and a lateral field with usually four incisures. The somewhat conically shaped lips are slightly offset, and there is no cephalic framework. The body is usually at least slightly curved, and it is C-shaped in death. The vulva is located about 83% of the body length, and the ovary is single. The tail, equalling from one sixth to one eighth of the body length, is ventrally curled and pointed. The procarpus and metacarpus are fused. Gravid females can become quite fat. Juveniles and males resemble females in body shape, but males and J4 have a greatly reduced stylet and esophageal musculature. (Dropkin, 1989; Mai and Lyon, 1975; Garaert, 1965; Jenkins and Taylor, 1967).

**Life Cycle.** Egg to egg generation time for P. projectus on ryegrass seedlings on 1% water agar at 18 to 20 C was 36 to 38 days (Woods, 1973). The females lay one to three eggs per day, and the eggs are in clusters due to sedentary feeding. Eck (1970) observed one female that laid 16 eggs in 7 days. Eggs hatch after seven to

eight days (Woods, 1973). The first of the four molts occurs in the egg, and J2 and J3 feed ectoparasitically on epidermal and root hair cells, while J4 do not feed (Rhoades and Lindford, 1961a). J4 accumulated in old pot cultures, but the stage was passed through quickly in young pot cultures, lab cultures, and some field soils. This stage was more tolerant of dessication and sudden exposure to low temperatures. These authors observed long term P. projectus and P. dianthus survival in moist stored soil. Almost all P. hamatus in fig root and soil samples were immature forms lying dormant in the soil (Throne and Allen, 1950). The molt from J4 to adult did not occur in the absence of root diffusates (Rhoades and Lindford, 1959; Dropkin, 1989), and Eck (1970) observed stimulation of P. projectus J4 molting by Iris germanica root diffusate.

**Host Range.** The first evidence of plant pathogenicity by a member of the genus Paratylenchus was reported by Lownsbery et al., (1952), who confirmed P. hamatus as the cause of extreme chlorosis of celery. The disease severity was proportional to the P. hamatus population size. Paratylenchus macrophallus was reported in mint tissue in Oregon by Horner and Jensen (1954). Jenkins and Taylor (1956) observed P. dianthus around carnation roots and suggested that this species



contributed to poor growth of the carnations. P. projectus was associated with decline of pasture grasses and in tobacco crops that followed (Coursen and Jenkins, 1958). P. epicris and P. minutus have been reported from the roots of black walnuts (Jenkins and Taylor, 1967). Paratylenchus spp. are parasitic on carrots, loganberries, raspberries, and English holly. Eck (1970) confirmed P. projectus pathogenicity on Iris germanica. Rose, apricot, peach, and all selections and hybrids of Prunus cerasifera are hosts for P. neoamblycephalus (Braun and Lownsbery, 1975).

**Behavior and Feeding.** Root penetration was achieved by P. projectus with one stylet thrust per second against the epidermal cell wall, interspersed with inactive periods of a few seconds (Eck, 1970; Wood, 1973). About 30 minutes elapsed before metacorporeal pulsation began. Rhoades and Lindford (1961b) reported that saliva flowed forward and filled the salivary duct and ampulla during this time. No flow of saliva from the stylet was observed, but the salivary reservoir became less opaque, and granular material aggregated near the stylet tip inside the cell (Rhoades and Lindford, 1959; Eck, 1970). P. projectus fed ectoparasitically, sometimes remaining at one site for up to seventeen days (Eck, 1970).

Most P. projectus fed on the early maturation zone of lateral roots and caused a decreased elongation rate (Eck, 1970). Feeding on root hairs was not observed by Wood (1973). Rhoades and Lindford (1961a; 1961b) observed ectoparasitic epidermal cell feeding and root hair feeding by P. projectus and P. dianthus. P. neoamblycephalus also fed ectoparasitically but with heads embedded in roots as deep as the cortex (Braun and Lownsbery, 1975).

Densities of Paratylenchus sp. are greater in fine-textured soils (Ferris and McKenny, 1975; Pinkerton, 1983; Dropkin, 1989).

**Disease Caused by Paratylenchus species.** During feeding, a dome of granular material formed within the cell around the orifice of the stylet tip (Wood, 1973). Apparently this mass caused little disturbance to the cell protoplast, since cytoplasmic streaming continued normally (Rhoades and Lindford, 1961b). Protoplasts of red clover root hairs contracted and disappeared after prolonged feeding, but these authors observed no other evidence of local pathology. P. neoamblycephalus occurred in clusters at ruptures in the epidermis and lateral root junctures of Myrobalan plum (Braun and Lownsbery, 1975). Parasitized roots were smaller and darker than normal roots and contained fewer feeder roots. P. hamatus produced shallow, localized lesions on Thompson seedless grapes

(Raski and Radewald, 1958). Tobacco exhibited stunting of top growth, shortening of internodes, and lack of characteristic large lateral roots when parasitized by P. hamatus (Coursen and Jenkins, 1958). Marked root proliferation in P. hamatus-infested plants resulted in an increase in root dry weight. Root weight of tall fescue parasitized by P. hamatus was increased, but shoots were stunted.

**Effect on Host Plant Yield.** In Scotch and native spearmint and in peppermint, P. hamatus retarded development and delayed flowering (Falkner, 1964). Fresh and dry weights of infected plants were reduced by 1-20% and by 20-36%, respectively. Eck (1970) found that root surface area of P. projectus-inoculated Iris germanica was significantly less than that of control plants and that size and vigor of inoculated plants was lower than that of controls. A suspension of P. neoamblycephalus inhibited root growth compared to uninoculated controls (Braun and Lownsbery, 1975).

Paratylenchus sp. populations on peppermint in western Oregon were positively correlated with both silt and hay yield in fields infested with Pratylenchus penetrans (Pinkerton, 1983). Heavier soils were less suitable for P. penetrans, and these soils had lower populations of this nematode and higher Paratylenchus sp.

populations. Therefore, higher Paratylenchus sp. populations correlated with low P. penetrans populations. P. hamatus densities were positively correlated with grapevine yield, growth, and vigor and perhaps even indicative of vigor (Ferris and McKenry, 1975).

**Population Dynamics.** New Zealand P. nanus populations around apple roots declined in winter and summer and increased in the spring (October) (Fisher, 1967). Numbers changed little between November and January except for a decrease in juveniles in October and an increase in females between November and January. Males always exceeded females, and juveniles always exceeded adults, but the proportion of females in the adult population increased from 27% in winter to 41% in summer. Females decreased in winter, increased in spring, and decreased again in summer. Numbers of P. hamatus on mint grown in greenhouse pots initially increased slowly but then increased rapidly until flowering, when numbers decreased (Falkner, 1964). Populations peaked again after flowering, often exceeding 100,000 per plant.

**CRICONEMELLA XENOPLAX**  
**AND OTHER CRICONEMATIDS**

Criconemella xenoplax Raski, 1952 is included in the subfamily Criconematinae Taylor, 1936 (Maggenti et al., 1988). The procarpus is fused to the metacarpus in members of the Criconematidae, a feature held in common with the Paratylenchidae. Females of C. xenoplax are 400 to 600 microns long and have a single ovary (Dropkin, 1989). Their stout bodies have a length to width ratio of 8 to 13.6 and taper little at the head and tail. The elevated lips are not offset, the tail is rounded, and the anus lies from the 4th to the 8th annule from the end (Raski, 1952; Dropkin, 1989; Jenkins and Taylor, 1967). The 71-86 micron stylet has prominent basal knobs. The ovary is single. Perhaps the most distinctive feature of these nematodes is their coarse, prominent annulations separated by deep divisions. Stylets and esophagi of males are degenerate (Dropkin, 1989), and males are known only from the type locality (Raski, 1952).

**Life Cycle.** The life cycle of C. xenoplax takes 25 to 35 days (Seshadri, 1964; Jenkins and Taylor, 1967). Females deposit eggs singly every two to four days, but

only after several days of feeding on roots (Thomas, 1959). The nematode is in the egg stage, in which the first molt occurs, for 11 to 15 days (Seshadri, 1964; Thomas, 1959). The J2 stage takes three to five days, the J3, four to seven, and the J4, five to six. Seshadri, 1964). The adult female spends two to three days in a preovipositional period (Seshadri, 1964).

**Host Range.** Woody perennials are good hosts for C. xenoplax (Seshadri, 1964), and Raski and Riffle (1967) found this species in soil around Pinus ponderosa, Juniperus monosperma, P. edulis, J. deppeana, and J. scopulorum in New Mexico. High populations have been observed on plum, apricot, grape, and almond, although strawberry, orchard grass, pear, cucumber, and alfalfa were not good hosts (Lownsberry, 1964; Lownsberry, 1961). C. xenoplax reproduced vigorously on two soybean varieties (McGawley and Chapman, 1983), on Rumex crispus, Lolium perennae, Vicia sativa, Trifolium incarnatum var. elatus, Vicia villosa, and Vigna unguiculata ssp. unguiculata (Zehr et al., 1986), and on leguminous weeds of peach orchards including three Lotus sp. and Cassia fasciculata (Zehr et al., 1990). Gramineous weeds in this study supported smaller increases, as did Portulaca oleracea and Geranium carolinianum. Sher (1959) documented the pathogenicity of C. xenoplax on carnation.

**Behavior.** Unlike most parasitic nematodes, C. xenoplax and other Criconematidae move using waves of elongation and contraction passing from head to tail (Thomas, 1959). The annules provide traction to aid in locomotion. Adults located favorable zones on host roots by trial and error probing with the head, and once the lips made contact, the stylet began to make short, deliberate probes while the head moved from side to side and up and down. The lips are held close to the region under attack. After a one to two minute interval, the stylet was inserted into the chosen cell, aided by muscles at the basal end of the stylet, and all other body movements ceased. Individuals have fed continuously for up to 18 hours in the lab, and they probably feed for longer periods under natural conditions. The long stylet may enable contact with cortical cells from outside of the epidermis (Jenkins and Taylor, 1967).

**Environmental Factors Affecting Populations.** C. xenoplax multiplied better in coarse soil than in finer soils around woody perennials (Seshadri, 1964; Lownsberry, 1964) and high populations have been documented on coarse soils around grass roots (Lucus, 1982). Jenkins and Taylor (1967) suggest that Criconematid locomotion is favored by lighter soils. Seshadri (1964) found that C. xenoplax multiplied well over a temperature range from 22

C to 26 C, although Lownsbery (1961) found that a soil temperature of 26 C favored increase of this species more than temperatures of 13 C, 18 C, 21 C, or 28 C.

C. xenoplax multiplied better at pH 7 than at either pH 3 or 5 (Seshadri, 1964), although Lownsbery (1961) observed no difference between population levels grown at pH 5 and pH 7 on peach. Both nematodes and plants grew best in soil at sticky point (15.5% moisture in the soil used) than at 3/4 or 1/2 sticky point (11.6% and 7.8%, respectively) (Shehadri, 1964). Jenkins and Taylor (1967) report that Criconematids are sensitive to low moisture and low pH and that this is consistent with their frequent recovery from bogs and from marshy soil. Criconemoides reedi and other Criconematids are found in cranberry bogs.

**Disease Caused by Criconemella sp.** Raski and Radewald (1958) observed no external symptoms from C. xenoplax parasitism on Thompson seedless grape roots, and Jenkins and Taylor (1967) suggest that the Criconematids are not strongly pathogenic. Free amino acid content in peach root tissue increased significantly with increasing C. xenoplax numbers (Nyczepir et al., 1990). Peach tree short life (PTSL) did not occur in the absence of C. xenoplax (Nyczepir, 1990). The gross appearance of infected roots of woody roseaceous perennials suggested that considerable damage was caused by this nematode



(Seshadri, 1964). In the presence of C. xenoplax, molar percentages of proline, glycine, and alanine in peach root cells increased, whereas arginine decreased (Okie and Reilly, 1984). They concluded that since this species is implicated in PTSL, it induced changes in root physiology to the point that normally non-lethal temperatures can cause tree injury. In addition, levels of the cyanogenic glucoside prunasin decreased in peach stem cuttings and seedlings, and prunasin increased in roots. Mitzutani (1980, in Okie and Reilly, 1984) suggested that breakdown of prunasin may be involved in PTSL, since it inhibits nitrate reductase upon breakdown to mandelonitrile and cyanide.

**Effect on Yield Alone and Interacting with Other Pathogens.** Carnation blooms and root systems were reduced and top growth was stunted by C. xenoplax and C. curvatum (Sher, 1959; Jenkins and Taylor, 1967). Preplant nematicidal soil fumigation improved the growth of peach replants (Lownsbery, 1959), and C. xenoplax added to peach rootstocks at planting reduced tree growth and increased susceptibility to disease caused by Pseudomonas syringae (Lownsberry et al., 1973). C. xenoplax nematode did not interact with Pythium sp. in the latter study. However, Lownsbery (1961) found that in lathhouse pot tests, peach seedlings were not injured by C. xenoplax at population

levels as high as any found to that date in California peach orchards. C. xenoplax populations were highest under the most vigorous peach trees and declined as tree vigor declined (Nesmith et al., 1981). C. xenoplax and Scutellonema brachyurum populations occupying the same root zones were inversely correlated with each other.

**EFFECT OF OXAMYL ON P. PENETRANS, PARATYLENCHUS SP.,  
CRICONEMELLA XENOPLAX, AND OTHER NEMATODES**

Oxamyl is a carbamate nematicide that is nematistatic at field application rates (Miller, 1970; Miller, 1972). Radewald et al. (1970) provided preliminary evidence that 1410 (oxamyl) suppressed reproduction by Meloidogyne incognita and Rotylenchus reniformis. Foliar applications of oxamyl of 4.79 kg/1000 l water, or less in some trials, controlled M. incognita on several hosts for 21 to 28 days. These sprays were prophylactic, and if oxamyl was applied after nematode infestation, their rate of development was reduced. Oxamyl was slightly phytotoxic in all foliage treatments proportional to the rate used. Harrison (1971) concluded that since hatched Heterodera rostochiensis J2 added to soil were able to develop in the roots of oxamyl-treated potato plants, oxamyl may interfere with egg hatch (Harrison, 1971). Oxamyl failed to kill 50% to 100% of eggs inside Heterodera tabacum cysts, yet little invasion of tomato roots occurred after application (Miller, 1970). Greater amounts were needed to kill unhatched than hatched J2. Wright et al. (1980) found that foliar applications of oxamyl reduced invasion by M. incognita juveniles but did not affect early stages of development of J2 already in roots. Potato root

diffusate from treated plants decreased hatching. To be effective, the nematicide had to be applied soon after plant emergence. Oxamyl's site of action is probably at the root surface or outer cortex, probably affecting nematode sensation (Wright et al., 1980).

Two foliar applications of oxamyl at two-week intervals dramatically reduced numbers of all stages of P. penetrans in peach, apple, and pear grown in growth chambers (Abawi and Mai, 1972). Rates of .91, .68, .45, or .23 kg ai per 378.56 liters water reduced P. penetrans root populations 95.2, 94.0, 89.7, and 47.8 per cent, respectively. Fewer nematodes penetrated roots, but there was no indication that nematodes left the roots of treated seedlings. More nematodes were found around the roots of treated than of untreated seedlings during the first three to four weeks after inoculation, suggesting that oxamyl in roots prevented P. penetrans invasion. This species was controlled in birdsfoot trefoil with soil drenches of oxamyl at 3.36 and 6.72 kg a.i./ha at seeding and foliar sprays of 1.9 and 3.8 g/l at four weeks after seeding (Willis and Thompson, 1973). Foliage and root yields increased in greenhouse studies. A soil drench of 3.36 a.i. kg/ha at seeding plus foliar sprays of 1.9 a.i. g/l at four and eight weeks after seeding reduced nematode

numbers by 99% at 16 weeks and increased foliage and root yields by 74% and 73% respectively compared to control.

Foliar applications of oxamyl prevented nematodes from invading the roots of diploid bananas (Gowen, 1977). One spray with 1.259 a.i. g/l was more effective than one, two, or three sprays with 0.625 a.i. g/l applied at five day intervals. When applied to nematode-infected plants, three 1.250 a.i. g/l sprays of oxamyl decreased nematode populations in roots. Foliar applications of oxamyl retarded infection of sugarbeets by Heterodera schachtii (Griffin, 1975). Maximum control was achieved when the nematicide was applied previous to or at the time of nematode inoculation. When foliar sprays of 0.04 kg a.i./100 l of water were applied to cabbage seedlings, oxamyl or a metabolite was apparently translocated to or became effective in roots within seven days. Under field conditions, oxamyl did not suppress development of Heterodera schachtii on roots during the first generation, but there was a large decrease in the second generation (Starr et al., 1978).

Foliar oxamyl treatments of table beets in growth chambers at inoculation reduced the number of developing cysts by 94% as compared to nontreated controls. These data agreed with other contemporary reports that foliar applications for control of Meloidogyne and Heterodera

were most effective when applied prior to nematode infestation and that application after inoculation significantly decreased the reproduction of these nematodes. Foliar sprays containing 3000 or 4000 a.i. mg/l applied before Meloidogyne hapla inoculation completely protected tomato plants from invasion for up to 36 days, while sprays containing 1000 or 2000 a.i. mg/l only partially protected plants (Stevens and Trudgill, 1983). Postinoculation sprays were less effective than preinoculation sprays yet still decreased the numbers of females and their developmental rate while increasing the number of males. Drenches or granules containing similar amounts controlled M. hapla more effectively than foliar applications, but there was a longer delay of nematicidal action after infection. Fewer larvae were killed, but more became males.

Oxamyl at 0.6-16.5 kg a.i./ha on peppermint produced a significant yield response (Pinkerton, 1983; Pinkerton and Jensen, 1983). Early spring foliar applications enhanced plant growth, while fall and summer treatments were ineffective, and applications of 1.1-2.2 kg ai/ha 5 to 10 days before spring flaming were recommended. Pinkerton and Jensen (1983) found that although March and November foliar applications produced greater yield response than did June applications, nematode populations

were only slightly and temporarily reduced by March and November foliar applications and did not correlate with yield response. Previously damaged plants were able to reestablish vigorous root systems during this reduction, and resume normal growth.

Eck (1970) found that tirpate and potassium azide can provide effective control of P. projectus in I. germanica plantings.

After August treatments with two nematicides, numbers of Cricinemella ornata were not reduced in all months but January and June in phenamphos-treated plots and were not reduced in fensulfothion-treated plots (Lucus, 1982). Numbers in fensulfothion-treated plots were higher than those in check plots in November and March following treatment.

## EXTRACTION METHODS AND THEIR EFFICIENCY

### INTRODUCTION

The extraction of nematodes refers to the separation of the desired animal from its medium. Nematodes are extracted for identification, to measure populations, and to facilitate recommendations regarding harmful species. Reliable and consistent nematode extraction is a prerequisite to other nematode studies. Some nematode life stages may not be accessible using the tools and methodology currently available. Extraction recovers only a proportion of those life stages which are captured by that procedure (Ferris, 1987). The process chosen should yield the highest number of the target species. Two or three procedures may be required for samples containing different species of life stages (Barker, 1985).

Soil extraction efficiency can be determined in different ways. A known number of nematodes may be placed in soil and the number of nematodes subsequently extracted compared to the number added. In contrast, an unknown number of nematodes may be extracted from intact soil and the attempt made to recover all those that are lost in different parts of the extraction process. Each of these two methods is a measure of extraction efficiency. Often, however, the numbers of nematodes recovered from two or



more methods are compared without reference to efficiency of any of the processes used.

## EXTRACTION OF NEMATODES FROM SOIL

### FACTORS AFFECTING ALL SOIL EXTRACTION METHODS.

Extraction efficiency is affected by the method and equipment used, soil type, sample volume size, nematode species and size, laboratory equipment and arrangement, time and personnel available, storage time, storage temperature, storage moisture, and the objectives of the population assessment. Seasonal shifts in the population, aggregation of the target species, vertical distribution, and migrational tendencies (Barker, 1985, Ferris, 1987).

**Storage time.** Some period of delay between sampling and extraction is inevitable. The length of this delay and the storage conditions of the soil during that time may affect the number of nematodes recovered. These results may, in turn, effect recommendations or management decisions. During storage, eggs may hatch, juvenile stages may molt, living nematodes may die, and dead nematodes may decompose. Thus, all these processes will affect recovery. However, in spite of the importance of storing soil before extraction, very little published research has sufficiently examined the effects of these factors.

Storage at room temperature or above may facilitate hatching of eggs, causing an apparent population increase, or accelerate lipid depletion and hasten starvation. Excessively cold storage temperatures (0 C or below) may kill nematodes. Very high or very low soil moisture during storage may reduce the number extracted, depending on that species' ability to withstand dessication and anaerobic conditions.

The composition of the population at collection time may influence apparent survival rates in storage. If large numbers of eggs are present, they may hatch during storage, especially at moderate temperatures, suggesting an increase in efficiency after storage. (Barker, et al., 1981). Heterodora juveniles and Paratylenchus and Pratylenchus spp. yields decreased after two weeks of storage and remained at that level until the fifth week (Emiligy and de Grisse, 1970).

Numbers of Xiphinema bakeri increased during the first month of storage at temperatures of 5 to 30 C. This was not due to hatching, however, since first through third stage juveniles decreased during this time. From one to six months, populations extracted contained more later developmental stages, and total numbers declined, especially at higher storage temperatures. (Sutherland and Ross, 1971).

In an evaluation of the direct centrifugal-flotation extraction method, fewer Pratylenchus sp. and fewer total plant parasites were recovered after five weeks in storage than after three weeks. No interaction was found between extraction method and storage time, except for *Dorylaimida*. Most *Dorylaimida* were recovered by the wet sieving/Baermann funnel method after three weeks; the fewest were recovered by Baermann funnels after five weeks (Dickerson, 1977).

Movement of endoparasitic nematodes from roots into surrounding soil during storage, increased the number of nematodes extracted from soil after storage (Kerr and Vythinlingam, 1966). Numbers of Pratylenchus spp. extracted from fall-collected soil samples may increase if stored at room temperature due to hatching and migration from root fragments in soil (Barker et al., 1981).

**Storage Temperature:** Storage temperature is one of the two most critical parameters that affect nematode survival during storage, the other being soil moisture. Storage at above room temperature resulted in rapid depletion of lipids, the main energy source for plant-parasitic nematodes (Barker and Campbell, 1981). Cooler temperatures suppress movement and reduce nematode metabolism, conserving energy reserves, and permitting survival in soil without host plants for long periods

(Babatola, 1981). Therefore, cooler storage temperatures are more likely to maintain greater survival and extraction efficiency.

Recommended storage temperatures vary among authors, but the optimal storage temperature for survival of most nematodes is 13 C. Meloidogyne incognita, Tylenchorhynchus claytoni, Belonolaimus longicaudatus, and Pratylenchus zeae densities remained constant or increased when soil was stored at 13 to 24 C for 16 weeks. This was also true for Helicotylenchus dihystrera and Xiphinema americanum extracted by Baermann funnels, whereas the numbers extracted by sucrose-flotation-sieving decreased slightly with time. All species except T. claytoni decreased appreciably in soil stored at 36 C. Low Baermann funnel recoveries of all species from soil stored at 2C may have resulted from chilling injury (Barker et al., 1969).

Storing soil at 31C to 35C for less than 1 week prior to extraction reduced the number of Pratylenchus loosi extracted compared to those stored at room temperature (Kerr and Vythilingham, 1966). These temperatures probably killed many nematodes, since returning the soil to room temperature for four days did not increase the number extracted. The optimal storage temperatures determined by ability to move through polymax filters were

15 C for Hemicyclophora arenaria, 3 C and 15 C for Pratylenchus scribneri, and 15 C for Trichodurus christei (Van Gundy and Thomason, 1962).

Nematodes can also be killed by cold temperatures. Exposure for 24 hours to -15 C killed 99% of Pratylenchus penetrans adults and juveniles and 75 to 90% of Tylenchorhynchus claytoni, Paratylenchus hamatus, Xiphinema americanum, and Rotylenchus robustus. Four hours at -9C killed twice as many P. penetrans as 24 hours at 4C. This endoparasitic species is more susceptible to freezing than are ectoparasites, which may be better adapted to sudden environmental changes (Miller, 1968).

Storage of nematodes in soil at -15 C for one to 16 weeks greatly increased nematode recovery by a sucrose-flotation-sieving method. All but Pratylenchus zeae and Tylenchorhynchus claytoni were killed by one week of exposure to -15 C. A marked increase in recovery of nematodes after these storage conditions may be due to partial dehydration of nematode cells and an increase in cell permeability. Nematodes injured in this manner would probably become plasmolyzed, and thus more bouyant, in sugar solutions. Extraction efficiency would then increase (Barker et al., 1969).

**Storage Moisture:** Soil which is too wet or too dry affects nematode survival. Excess moisture can result in

rapid decline of populations, thereby lowering yields from some extraction procedures. Low oxygen and relatively high moisture favor survival of eggs and/or juveniles of Meloidogyne javanica and Tylenchulus semipenetrans. Life stages that survive best under various moisture conditions vary with species (Barker and Campbell, 1981).

Hirschmaniella spinicaudata survived dessication better than H. oryzae or H. imamuri. Twenty seven per cent of H. spinicaudata survived for ten months in dry soil. In the absence of rice, H. oryzae survived best in wet soils. H. spinicaudatus may survive dessication better than the other species because of its slower dessication rate. Adults of the latter two species survived better than second stage juveniles at all relative humidities tested (Babatola, 1980).

**DYNAMIC VERSUS MECHANICAL EXTRACTION METHODS:** Soil extraction methods fall into two broad categories. Dynamic methods are procedures relying on the activity of the nematodes, most of which are Baermann funnel derivations. Mechanical methods are those that use physical processes to extract nematodes (Murphy, 1962).

**DYNAMIC SOIL EXTRACTION METHODS.** Baermann originally devised his funnel extraction system to separate hookworm larvae from fecal cultures and contaminated soil (Murphy, 1962). The method also effectively recovered Turbellaria,

Rotifera, Tardigrada, and Enchytraeidae. Initially, Baermann loosely tied a piece of cheesecloth around a soil sample and suspended this in a funnel of water just under the water surface of a water-filled funnel.

Yields reported were consistently low, although Pratylenchus sp. and free-living forms were extracted somewhat better than other nematode groups. As described, it yielded about 12% of the total nematode population compared to that obtained by the flotation and cottonwool methods (Oostenbrink, 1960). The original description is rarely if ever followed today, and currently used methods are properly designated as modified Baermann funnels. However, for simplicity, they will be referred to herein as Baermann funnels. In a modified Baermann funnel procedure, soil is placed on filter material supported by a sieve suspended in the top of a water-filled funnel. A flexible tube is placed over the stem of the funnel and is closed off by a clamp or screw clip. After a specified extraction period, nematodes which have migrated into the water and settled to the bottom of the tube are drained off.

Baermann funnels have several advantages over other extraction methods. They are simple and quick to use (Chapman, 1958), they extract only live nematodes capable of movement (Hooper, 1986), so only infective nematodes



are counted, and they are especially suited for collecting minute juveniles of species such as Paratylenchus and Tylenculus (Thorne, 1961). This method has been reported to be the most efficient for a few genera, such as Pratylenchus (Barker et al. 1969).

Baermann funnels may also be used for large scale inoculum collection. Van der Vecht and Bergman in 1954 constructed a Baermann funnel four feet in diameter at the Bogor, Java, experiment station. By draining off one liter of water, they were often able to collect over one half million Radopholus oryzae for use as inoculum (Thorne, 1961).

**Sample Volume:** Sample volume affects numbers extracted. Densities of Helicotylenchus dihystrera and Meloidogyne arenaria recovered from Baermann funnel extractions of 50, 100, 200, or 400 g samples were significantly different but were not directly proportional to the relative volume of soil used (Rodriguez-Kabana and Pope, 1981). Significantly fewer Hoplolaimus galeatus were recovered after 24 hours from the two larger sample sizes than from the two smaller. Seinhorst (1962) found Baermann funnels reliable only when a very thin layer of soil was spread on the sieve, since nematodes become inactive soon after being immersed. Mist chamber extraction of soil, in which soil on filter material

supported by a screen in the top of a funnel was misted with warm water at frequent intervals, was thus recommended (Seinhorst, 1962).

**Nematode species Characteristics.** Characteristics of the target species, such as population-age structure, activity and motility, and size and ornamentation of the nematode, affect dynamic processes (Ferris, 1987). In addition, there may be biotic or abiotic factors in the soil sample that are detrimental to the nematodes or that either positively or negatively affect their activity.

**Funnel Filter Material.** The filter material used affects recovery from Baermann funnel extractions. Some materials, in their role of retaining soil, sievings, or other substrates can greatly inhibit the passage of nematodes. Materials which have been used include various brands of facial tissue (Rodriguez-Kabana and Pope, 1981; Barker, et al., 1969, Walker and Wilson, 1960), toilet tissue (Viglierchio and Schmitt, 1983), and silk bolting cloth (Staniland, 1954). Nematode recovery varied between nematode species and between tissue type and brand (Viglierchio and Schmitt, 1983). For example, of eight toilet tissue brands tested, MD alone allowed the highest recovery of Meloidogyne incognita, while both MD and Truly Fine allowed comparable Ditylenchus dipsaci recovery. Davis Lumber tissues allowed the second highest level of

D. dipsaci recovery but was one of the least effective tissues in M. incognita recovery.

**Soil Compaction:** Recovery from Baermann funnels may be reduced by compaction during soil probe sampling if nematodes occupying soil macropores are either crushed or immobilized (Kimpinski and Welch, 1971). Compacted clay yielded significantly fewer nematodes than non-compacted clay when extracted in Baermann funnels but almost equal numbers when processed by sucrose flotation. However, sand, which compacts little, gave similar yields with either technique.

**Storage Time:** Storage time (discussed above) affects the efficiency of all extraction procedures, but it is perhaps most noticable and consequential for dynamic extraction processes. Storage temperature, also mentioned above, is also a special consideration for Baermann funnel extractions, since the extraction depends on the motility of the nematodes, and sucessful extraction requires nematodes to be alive and motile for extraction.

**Extraction Temperature:** Extraction temperature influences the proportion of the nematode population recovered. A generally stated optimum is between 15 and 25 C (Murphy, 1962; Adams, 1965). Large differences have been reported in nematode numbers recovered from the same soil samples extracted at different temperatures (Adams,

1965). Highest recoveries were at 18 C of Pratylenchus sp., at 5C for Paratylenchus sp. females, 25 C for Paratylenchus sp. juveniles, at 20 C for Aphelenchoides sp., and at 30 C for saprophagus. In Baermann separation of Cobb sieved soil samples, different Xiphinema species showed different temperature optima at which yield could be at least two times that at other temperatures (Flegg, 1967). Similarly, Kerr and Vythilingam (1966) also observed a marked yield reduction for Pratylenchus loosi at 27-35 C compared to room temperature.

Nematodes respond to temperature gradients, moving away from room temperature soil to cooler regions (Robinson and Heald, 1989). Loosely covering the material being extracted in funnels reduces the temperature gradient caused by evaporative cooling and thus increases extraction efficiency over that in uncovered funnels. Increased numbers of Meloidogyne incognita, Tylenchus semipenetrans, and Rotylenchus reniformis were obtained after 2.5, 5.5, and 24 hours, usually by one to two orders of magnitude. Open funnels never yielded quantities of M. incognita or T. semipenetrans comparable to covered funnels. Open and covered funnels yielded comparable quantities of R. reniformis after 48 hours.

**Extraction Duration:** Various researchers cite different extraction durations. Overnight extraction is

common (Staniland, 1954, Christie and Perry, 1951). Periods of 2.5, 5.5, and 24 hours were used by Robinson (1989). Various workers recommend 24 hours (Walker and Wilson, 1960; Rodriguez-Kabana and Pope, 1981), 24-48 hours (Hooper, 1986a), 48 hours (Robinson and Heald, 1989), three days (Barker, Nusbaum, and Nelson, 1969) and four days (Anderson and Yanagihira, 1955). Yields of Xiphinema sp. increased markedly with time over the first 20 hours but few additional nematodes were recovered thereafter (Flegg, 1967).

Periodic removal and enumeration of nematodes extracted during the Baermann funnel procedure suggested appropriate extraction durations. The extraction rate for Helicotylenchus dihystrera and Meloidogyne arenaria was highest in the first 24 hours, but thereafter, the rate of cumulative increase was almost constant (Rodriguez-Kabana and Pope, 1981). The extraction rate for Hoplolaimus galeatus varied little during the first 72 hours for most samples and then declined between 72 and 96 hours. The mist chamber-funnel method recovered most Ditylenchus after 72 hours with 80% extracted after 21 hours (Oostenbrink, 1960). However, in the same experiment, Meloidogyne extraction was not completed after two weeks. Eighty percent were extracted after six weeks but only 10% after one week (Oostenbrink, 1960).

**Problems with Baermann Funnel Use:** Several problems have been encountered in Baermann funnel use. Funnels can occupy considerable space (Anderson and Yanagihira, 1955). Oxygenation is poor, especially at the base of the funnel where nematodes collect (Hooper, 1986; Flegg, 1967). Flegg observed that extraction by suspending soil in water in a flat dish yielded more active nematodes, but samples were dirtier and yields were lower than those from Baermann funnels. Baermann funnels were thus used due to higher yield. Funnel techniques are not efficient for some less motile genera, such as Criconemoides, Helicotylenchus, Hemicriconemoides, and Macroposthonia (McSorley and Parrado, 1982, Barker and Nusbaum, 1971, Walker and Wilson, 1960). Chapman (1958), using 90 mm diameter 1.0 mm sieves, found Baermann funnels limited to small amounts of soil, since efficiency decreased when sample size was greater than 20 g. Reliable quantitative work was impossible with small samples, however, because yields were low and variation was high. In a modified Oostenbrink cottonwool variation of Baermann funnels, Townshend (1963) used soil samples up to 150 g on filters supported by cheesecloth in 18 cm embroidery hoops. Dickerson (1977) in his comparison of extraction methods, found Baermann funnel extraction the most inconsistent. The debris in the sample water varied and sometimes made

counting impossible. The effectiveness of Baermann funnels can be affected by the time of year, and low recoveries in winter may reflect decreased nematode motility (Barker, et al., 1969). Innovative solutions to these and other problems have helped to refine funnel techniques and to prompt the development of new methods.

**Modifications of Baermann Funnels:** The initial modification was to replace the cloth bag that Baermann used with a screen to support a filter holding the soil or plant material. Other innovations included containers of plastic pipe with screen cemented inside (Walker and Wilson, 1960), and wire mesh soldered to copper pipe, the mesh supporting silk bolting cloth (Staniland, 1954). All of these support mechanisms were then set into water-filled funnels to complete the extraction.

To solve their space problem, Anderson and Yanagihira (1955) coated the inside of conical paper cups with paraffin and suspended soil samples in "Scotties" tissues. After four days, the bottom of the cup was punctured, and 12 to 15 ml of water was drained; cups were disposable. Poor oxygenation in funnels was improved by the use of oxygen-permeable polyethylene tubing in place of the traditional rubber tubing (Hooper, 1986).

Oostenbrink, (1960) developed a cotton-wool extraction method in which a soil suspension is poured

onto a double cotton-wool filter supported by a coarse mesh sieve. Townshend (1963) adopted this procedure to develop a system in which soil was placed on cottonwool filters supported by cheesecloth stretched over plastic embroidery hoops placed in 19 cm wide aluminum pans so that soil was barely immersed. The pans were stacked and covered by a plastic hood. Water from an aerated source was frequently changed. This alleviated both the space and the aeration problems (Townshend, 1963a).

Stoller (1957) used a larger diameter funnel to accomodate a larger sample. Oxygen-permeable plastic tubing was attached at the bottom, and extracted nematodes were stored in this tubing by closing off each end of the tubing and removing from the funnels. Sample size and aeration problems were addressed, and the system cost less than others in use.

There are few references to the amount of water drained from Baermann funnels. Standiland (1954), attached a capillary tube to the rubber tubing, which was closed by a screw clamp. To retrieve the nematodes, the clamp was unscrewed slowly enough to exude a small drop of water replete with nematodes through the end of the capillary tube (Standiland, 1954).

Baermann funnel extraction efficiency has been increased by the use of detergents or flocculants. In



extracting juveniles of cattle-parasitic nematodes from pasture vegetation, Rohrbacher (1957) found that 0.5 ml Triton X-100 detergent/l had no apparent effect on the viability of juvenile nematodes but almost doubled the number of nematodes recovered. This was probably due to better wetting of the vegetation. Although free-living nematodes were not counted, there appeared to be more (Rohrbacher, 1957).

**MECHANICAL EXTRACTION METHODS.** In contrast to dynamic extraction methods, in mechanical methods, the role of the animal is passive. However, "mechanical" methods is a euphemism: these methods rely mostly on the labor of the operator, not on mechanical equipment (Murphy, 1962). Mechanical methods exploit the different sizes and sedimentation rates of soil particles and nematodes. Mechanical methods used to separate soil animals from soil include direct examination, sieving procedures, sedimentation, elutriation, and various flotation techniques with and without wet sieving and/or centrifuging (Murphy, 1962).

Direct examination involves hand sorting of specimens from their medium or suspending soil in water and picking the animals out. The efficiency depends on sample size, animal density, and operator skill, patience, and

perseverance. Currently, this technique is mainly limited to earthworms (Murphy, 1962).

Minderman (1956) described a lactophenol cotton blue staining method which enabled direct examination of nematodes in the soil. However, while this is valuable for checking the reliability of other techniques, it is too time consuming to be used routinely.

**Factors Affecting Mechanical Procedures: Sieve Size.**

Sieves are used in almost all mechanical extraction methods, and the choice of sieve size can greatly effect extraction efficiency. Large mesh sieves (approximately 5-8 mm) are used for initial screening of soil to break clods and facilitate sample mixing. Intermediate mesh sieves (approximately 1-2 mm) are used to separate larger objects, such as organic debris and gravel and large sand, from soil suspended in water. Fine mesh sizes (55 u and smaller) concentrate nematodes suspended in liquid from which most sediment has been removed by settling, centrifugation, or turbulence processes. The sieve(s) used should retain all stages of nematodes without too much debris, and this condition is difficult to achieve when extracting large nematodes (Flegg, 1967).

Recovery is a function of sieve pore size and nematode body size. Whereas sieves of 45, 38, and 25 u (325, 400, and 500 mesh) are often used for final

separation, representative nematode body diameters are 12 u (Tylenchulus semipenetrans juvenile), 15 u (Meloidogyne sp. juvenile), and 25 u (Xiphinema americanum adult).

Thus, only nematodes whose bodies fall at an angle across the sieve wires are retained, and even some of these wash through with continued water passage (Ferris, 1987).

Hooper (1986) found sieves of 45-53 u apertures to be in general use for screening nematodes from soil suspension, since smaller apertures tend to become clogged with soil. However, there is considerable loss even when using a 25 u sieve (McSorley and Parrado, 1981).

Therefore, Hooper recommended the use of 10 or even 5 u sieves of monofilament nylon when possible. Many sieve size combinations are probably adjusted for local soil types.

One disadvantage of sieving methods is that nematodes can be lost by passing end-on through sieves or be damaged by being bent across the sieve wires. The use of a partially submerged sieve can decrease the chances of such damage (Hooper, 1986).

Miller (1957b) advocated wet sieving of nematode samples to decrease volume instead of the widely accepted decanting and settling procedure. In this procedure, a suspension of soil and nematodes is left for a specified time to allow settling of soil particles while nematodes

remain in suspension. Miller reported that more of the nematodes extracted were retained on fine mesh sieves after wet sieving than after decanting and settling.

McSorley and Parrado (1981) used a combination of sieving and extraction procedures to compare the efficiency of recovering nematodes from soil suspensions using different mesh sizes. Comparing 53, 45, or 38  $\mu$  sieves, no difference was seen in numbers of larger species (Helicotylenchus and Criconebella) recovered. However, 38  $\mu$  was the most efficient sieve size for recovering Rotylenchus reniformis juveniles. Openings of 25  $\mu$  became clogged, and recovery was poor with the soil type used.

The same researchers investigated the decanting of nematode-containing sucrose solutions onto different sized sieves. Helicotylenchus was unaffected by mesh size, but 25  $\mu$  was the most efficient for R. reniformis juveniles. Rinsing of the sieve to remove sucrose caused significant loss ( $p = .05$ ) of R. reniformis. Therefore, the choice of sieve size is critical in obtaining quantitative data on small plant-parasitic nematodes (McSorley and Parrado, 1981).

The sieve dilemma is avoided altogether with the originally described centrifugal flotation technique (Caveness and Jensen, 1955). Soil is introduced directly

in water, and the final supernatant is reduced by settling and decanting. However, the sample size is limited to 50 cc of soil.

**Sedimentation Rate:** Sedimentation rate is the time taken for a nematode to fall through a given distance in a liquid. Different settling rates of adults and juveniles influence the choice of sedimentation time (Flegg, 1967). Viglierchio and Schmitt (1983) approximated the sedimentation rates of various nematodes through water in a sedimentation column designed to eliminate motion and thermal convection. Nematode density, differential drag due to wriggling, and undetectable thermal gradients affected sedimentation. Ferris (1987) concurred, stating that activity may influence flotation in processes that do not require nematode motility. Vilierchio and Schmitt (1983) also found that as the heterogeneity of a nematode population increased, so did the variability of the sedimentation rate of individuals. The range of sedimentation rates for a concentrated population also increased with increasing heterogeneity.

**Sieving Methods:** Soil sieving includes both wet and dry methods. The latter is used primarily for extracting arthropods. Wet sieving of soil to extract nematodes was first used by Cobb in the early 20th century (Murphy, 1962b). A liquid suspension of soil was poured through a

bank of sieves of decreasing mesh size (e.g., 1000 u progressing to 56 u or finer) and agitated. Cobb found that sieving was faster and damaged fewer nematodes if the difference between adjacent sieve mesh sizes was small (Murphy, 1962).

In contrast, Thorne (1961) admonished that sieves should be used singly and recommended that no attempt should be made to superimpose them and work the sample through simultaneously. However, in a modification of the method, Xiphinema and Longidorus spp. were collected on a bank of 106 u sieves rather than on single sieves of a range of apertures (Flegg, 1967).

In an examination of the efficiency of the Cobb sieving method (Townshend, 1962), the percent of Pratylenchus penetrans extracted from fine sandy loam varied with the weight of the soil sample, the size of mineral particles, and population density. The lowest percentage of Xiphinema americanum, P. penetrans, and P. hamatus were recovered from fine sandy loam, followed in order by clay, sand, and water. Percentages of the large nematode X. americanum extracted were greater than those of either Pratylenchus species.

Thorne (1961) found that failure to use sieves efficiently was responsible for the wide use of Baermann funnels, which generally gave less satisfactory results.

The efficiency of modified Cobb sieving followed by final extraction in Baermann funnels was 86 + or - 3% and 90 + or - 5% for two Xiphinema spp. When compared with elutriation, in which nematodes and soil are suspended in water and separated by density using high turbulence and by size by sieves, Cobb sieving produced a cleaner suspension with more active nematodes. However, sieving generally takes more operator time than other methods, such as Baermann funnels, and it does not separate live from dead nematodes (Anderson and Yanagihira, 1955).

**Christie and Perry Method:** The Christie and Perry extraction method combines dynamic and mechanical procedures, as do several methods which further process mechanically derived samples by Baermann extraction. The dilemma of finding neither Cobb sieving nor Baermann funnels adequate to provide clean samples was the impetus for the method's development (Christie and Perry, 1951).

In this procedure, soil is stirred in water and left to settle for a few seconds. The suspension is decanted through two sieves - 670 to 800 u and 80 to 106 u - and the water retained. Resuspension and decanting is repeated twice, and the material in the coarse sieve is washed with fine spray while over the fine sieve, and then the material in the fine sieve is sprayed to remove colloids. Fine screenings are poured onto a filter

resting on the surface of the water in a filled Baermann funnel. (The original filter material was silk bolting cloth.) After five minutes, the funnel clamp is opened to drain a few milliliters of water containing nematodes into a counting dish or sample vial. Funnels were usually set up in late afternoon and samples collected the next morning.

Thorne (1961) found the Christie and Perry method superior to both the Baermann funnel method and to centrifugal flotation in both efficiency and ease. Soil samples of up to 400 g were processed by Chapman (1958), and even larger samples could yield reliable results if the initial suspension was stirred mechanically. This method yielded the most active nematodes and similar recovery to centrifugal flotation with three or more centrifugations using zinc sulfate (Bravo, 1977).

Some researchers have found this method cumbersome and time-consuming. Although large soil samples can be processed, without yield reduction, large volumes of water (8 to 10 quarts) must be handled (Chapman, 1958). As with simple Baermann funnel extraction, this method is inefficient for sluggish genera such as Criconemoides, Helicotylenchus, and Macroposthonia (McSorley and Parrado, 1982). However, the method does compare well with a centrifuge technique modified from Jenkins (1964) for



Rotylenchus and Meloidogyne. According to several authors, efficiency of the Christie and Perry method is greatly influenced by temperature and soil moisture and therefore unsuitable for measuring seasonal fluctuations in field populations (Bravo, 1977). Again, this agrees with observations about Baermann funnels in general.

#### **METHODS INVOLVING SEDIMENTATION AND SIEVING.**

Seinhorst (1962) found that the diameter of soil and organic matter particles having the same sedimentation rate as nematodes of a given size will be less than two thirds the size of the sieve mesh that will retain those nematodes. However, soil particles retained on a sieve of a given mesh will have a sedimentation rate of about twice that of the smallest nematodes retained on that sieve. Therefore, sedimentation in water will separate nematodes from soil particles having a greater sedimentation rate, and sieving will separate nematodes from those particles having the same rate of sedimentation.

**Inverted Flask:** Sedimentation without a counter current (as used in elutriation methods) may be used to separate nematodes from small soil samples (Seinhorst, 1962). Sedimentation techniques may be used following elutriation, since nematodes settle more quickly than soil particles of a given size. Seinhorst's (1955) two-flask

sedimentation method recovers 60 to 75% of the nematodes in the sample (Murphy, 1962).

Seinhorst's two-flask method gives a cleaner extract than that usually obtained by direct sieving (Seinhorst, 1955, cited in Hooper 1986). In this process, a 200 g soil sample is dispersed in 750 ml water and the mixture washed through a 2 mm sieve into a large, wide-stemmed, stoppered funnel. After all soil has passed through the sieve, the funnel plug is pulled and the suspension allowed to run into a 2 l Erlenmeyer flask. A short, 12 mm diameter funnel is attached to the top of the flask (flask A). The soil suspension is agitated and inverted over a similar flask filled with water (flask B). After ten minutes, flask A is inverted over a beaker (beaker A) and flask B, with funnel attached as on flask A, is inverted over another beaker (beaker B). After ten minutes, flask B is inverted over beaker B. The soil suspension should be agitated before each inversion. After the three ten minute periods, the contents of flasks A and B are poured through a 53 u sieve, and the contents of beaker B are poured through a 90 u sieve. Beaker B contains almost no nematodes and the contents are therefore discarded. This process provides a relatively debris-free suspension in a relatively short time. Chapman (1958) allowed three minutes for the first

settling and two for the second. Soils containing more clay required four to five minutes. Extracts from clay soils were clear, but yields were lower than with other soil types (Chapman, 1958).

**Seinhorst Sedimentation Apparati.** Seinhorst (1956) described an assembly of flasks, tubes, and other glassware through which a continuous flow of water is maintained at different speeds to separate nematodes from soil particles. Flow rates are adjusted according to the relative size and density of nematodes and particles. Suspensions in the sample are passed through sieves of different sizes five to seven times in a continuous flow system. Gravity and water current separates nematodes from soil, whereas current speed and mesh width determine the size of nematode recovered. Water flowing 230-350 cm/hr recovers Ditylenchus sp., Pratylenchus sp., and Aphelenchoides sp., whereas water flowing 600-700 cm/hr recovers Hoplolaimus uniformis, Mononchus sp., Dorylaimida, and Criconemoides sp., and water flowing at 1400 cm/hr recovers large Dorylaimida. Recovery of a particular nematode species depends on nematode size relative to the sieve mesh size, suspension volume, and duration of sieving. Sixty five per cent of Pratylenchus penetrans adults (.6 mm in length) were retained on the sieves but only 25% of juveniles (.3 mm). Seventy five

per cent of Ditylenchus (.8 mm) and almost 100% of Hoplolaimus adults (1.5 mm) were retained (Seinhorst, 1956).

Per cent recovery is very nearly expressed by:

$$100(1 - ax)$$

where a = fraction of total number of nematodes of a certain length present in the suspension which passes through the tube, and x = the number of times a sample is sieved (Seinhorst, 1956). This is probably the most accurate apparatus for nematode extraction (Thorne, 1961). Final extraction in Baermann funnels is recommended to separate nematodes from remaining debris (Seinhorst, 1956). The soil sample cannot exceed 500 g, which, as Thorne (1961) pointed out, is a small portion of a field plot, but does compare favorably with sample size maxima of other techniques.

Trudgill et al. (1973) developed an apparatus similar to that of Seinhorst. A 38 cm long 63 mm diameter column of perspex tubing is sealed by O rings into another tube containing two removable plastic sinters, below which water is introduced. A cone at the top supports a 10 cm diameter 1400 u aperture sieve, on which soil is placed. Flow rate, determined by the relationship between the mean diameter of the nematode and its settling time, is monitored by a flow-meter. Nematodes and other particles

that rise to the top and overflow are directed into sieves by a perspex collar. The water volume of about two liters is designed for 200 ml soil samples. This method is especially effective for Heterodera cysts and white females.

**Elutriation:** Oostenbrink (1954, 1960) designed the elutriator in 1954 and modified it in 1960 (Murphy, 1962). A 200 g soil sample placed on a 1000 - 4000 u sieve at the top of a funnel is sprayed with a constant flow of water. The funnel drains into a conical vessel which is filled with water before beginning the process. A countercurrent of constant flow enters from the bottom of the conical vessel at the same rate with which the soil is sprayed from above. Soil settles to the bottom of the apparatus and is emptied by opening an aperture at the bottom. Nematodes separated from heavier soil particles pass out a tube near the bottom of the vessel onto sieves (Murphy, 1962) or into vessels for other methods of nematode separation (Hooper, 1986a).

Seinhorst (1956) designed a five stage elutriation system which was modified by Seinhorst (1962) and Murphy (1962). In Seinhorst's 1962 modification, soils are dispersed in water (especially those heavy in clay) before placement into the elutriation vessel using a motorized "vibromixer", which reportedly does not harm the

nematodes. An upcurrent of water separates nematodes from soil particles and holds them in suspension while heavier particles sink. After elutriation, Seinhorst (1962) poured the extracted nematode suspension onto filter paper, and a significant portion of the population of nematodes is retained on the paper (Seinhorst, 1962). Murphy (1962) used Baermann funnels following elutriation to further separate nematodes from remaining debris.

Many extraction methods based on sedimentation and sieving have been described, but most require too small a sample or too much intensive labor to be practical for routine sample processing. The elutriation method, however, can accomodate a relatively large sample (100 to 1000 ml) yet requires less labor than other methods (Seinhorst, 1962). Some designs allow the operator to extract several samples at one time.

However, while elutriation techniques are quantitatively satisfactory, other researchers found that they are more laborious than other methods relying on sedimentation and screening (Hooper, 1986a). For example, more nematodes were obtained by elutriation than by Baermann funnel extractions, but elutriation took about twelve times as much of the operator's time (Walker and Wilson, 1960). In addition, the suspension produced by

the elutriator contained more debris than that from modified Cobb sieving (Flegg, 1967).

Sedimentation rates of nematode species and of individuals within a species can vary (Viglierchio and Schmitt, 1983). Therefore elutriation, which uses high rates of turbulent flow, may have some merit in separating light organic particles from dense mineral particles, but its use in quantitative nematode separation is open to question.

**Density Flotation: With Flocculants, With Centrifuge, or Direct.** Nematodes can be extracted from soil or organic debris by floating them out in a solution with a specific gravity greater than their own. Flotation is recommended for extracting sluggish species such as *Criconeematiods* (Hooper, 1986). Procedures using wet sieving followed by flotation are also used for arthropod eggs, larvae, and pupae as well as for nematodes (Murphy, 1962).

**Choice of Solution:** A solution with specific gravity between that of the target organisms and accompanying soil particles is needed for adequate separation. Sucrose is often used, since it is inexpensive and readily available (Hooper, 1986). Magnesium sulfate solution is attractive, since it lacks sucrose's stickiness. Zinc sulfate has less osmotic effect than either sucrose or magnesium

sulfate solutions of similar densities but is more acidic and toxic (Hooper, 1986a). Magnesium sulfate is not recommended for morphological or taxonomic work, since specimens extracted in this solution are of poor quality. Internal organs are severely affected, outlines are vague, detailed structure is lost, and the esophagus is obscure (Decraemer, Coolen, and Hendricks, 1979).

Sucrose, magnesium sulfate, and Ludox LS, a colloidal solution of silica, were compared for density centrifugation extraction of Trichodorus primitivus from sandy loam soil by Decraemer, Coolen, and Hendrickx (1979). Mean recovery with Ludox LS was 275, with sucrose, 250, and with magnesium sulfate, 225. Ludox LS is the only liquid with which Longidoridae can be extracted in the same operation as the smaller species.

Good results have been obtained using molasses as a low-cost density flotation solution. In paired comparisons, a molasses solution extracted more plant parasitic, dorylaimid, mononchid, and other soil nematodes than did a sucrose solution of the same specific gravity, which was attributed to its higher viscosity (Rodriguez-Kabana and King, 1975).

**Density of Solution:** In their original description, Caveness and Jensen (1955) recommended a sucrose concentration of 484.5 g/l of water. Thistlethwayte and



Riedel (1969) determined that the specific gravity (sp. gr.) of this solution was 1.14, not 1.18 as stated in the original description. A sp. gr. of 1.18 requires 673 g/l of water, or 475 g/l of solution. The sp. gr. of nematodes is between 1.04 and 1.09, markedly lower than 1.14, and Caveness and Jensen (1955) found solutions as low as 1.10 sp. gr. satisfactory for nematode extraction. Of 570 Ditylenchus dipsaci added to soil, 291 were recovered with a 1.10 sp. gr. solution, 289 with 1.14, and 264 with 1.18. More Criconemoides and Helicotylenchus were obtained with 618 g sucrose/liter solution than with 454 g/l or 1135 g/l, but different concentrations had no effect on recovery of Hoplolaimus (Weber and Williams, 1968). Thistlethwayte and Reidel (1969) and Jenkins (1964) reported success with sucrose solutions of 454 g/l solution, which has a sp. gr. of 1.13, Dickerson (1977) used a sucrose solution with sp. gr. of 1.18 (673 g sucrose/l water) (Thistlethwayte and Reidel, 1969), and Minderman (1956) used magnesium sulfate of sp. gr. 1.25 (approximately 245 g/l solution) (Hooper, 1986). Byrd, et al. (1966) reduced the sp. gr. of the sucrose solution to 1.0 M with little loss in nematode recovery. This lower concentration resulted in less damage to small nematodes.

As sp. gr. increases from 1.00 to 1.20, nematode recovery increases, but the percentage of motile nematodes

recovered decreases (Vigliierchio and Yamashita, 1983). Therefore, it is prudent to use the lowest effective density solution of a solute producing the lowest osmotic activity (Vigliierchio and Yamashita, 1983), if viability of recovered nematodes is important.

**Cyst Extraction by Wet Sieving/Flotation.** Fenwick (1940) designed the apparatus now known as the Fenwick can for extracting nematode cysts from soil. Because moist cysts sink in water, the soil is air dried before extraction. It is then sieved through a 6.4 mm sieve and washed through further sieves into the flotation vessel. The flotation vessel, as modified by Oostenbrink is filled with water by a strong jet from above and is suitable for samples of up to 300 g. Organic material and some soil overflow onto collecting sieves, recovering about 70% of the cysts. A fast water flow from the bottom then carries cysts, which are still trapped in the flotation vessel, onto the sieves (Shepherd, 1986). The floating material collected on the sieve is washed further and transferred to filter paper for examination or further processing (Murphy, 1962). Various improvements upon this method have been devised and have been reviewed by Shepherd (1986).

**Centrifugal Flotation:** Caveness and Jensen (1955) described a centrifugal flotation technique for the

isolation and concentration of nematodes and their eggs from soil. Fifty cc of soil is divided among three centrifuge tubes, and 30 ml of water is added to each. The tubes are centrifuged at 4800 rpm for five minutes, and the supernatant is discarded. Sugar syrup (484.5 g/l water) is introduced, the pellet resuspended and the tubes centrifuged as before. The sucrose solution containing the nematodes is added to 500 ml water to avoid harmful effects of the sucrose, and the excess solution is decanted after 20 minutes of settling. Caveness and Jensen estimated the total number of nematodes in the solution by pouring it into a 1 liter graduate, adding water to 250 ml, and shaking. Nematodes in 10 ml of solution were counted.

Various researchers use different centrifuge speeds and times. Caveness and Jensen (1955) used 4800 rpm for five min for both centrifugations, while Jenkins (1964) used 1750 rpm for 4-5 min for the first, and 1750 rpm for 0.5 to 1.0 min for the second. Weber and Williams (1968) used 2600 rpm for four min and then for one min; Barker, Nusbaum, and Nelson (1969) used 420 G for five min for both centrifugations; Dunn (1971) used 1280 G for four min; Dickerson (1977) used 2,514 G for times varying from 4.0 to 6.0 min.

Caveness and Jensen (1955) reported that recovery of nematodes using centrifugal flotation is significantly greater than that from Baermann funnels or gravity screening. Eggs were not recovered using funnels or screening but were obtained using centrifugal flotation (Caveness and Jensen, 1955; Seinhorst, 1962). Harrison and Green (1976) found centrifugal flotation to be the most versatile extraction method of four tested and the best for use in clay soils.

Thorne (1961) observed that recovery was higher from the Caveness and Jensen method than from Cobb sieving but that sample size was quite small. He felt that none of Oostenbrink's suggested variations had any particular advantage over techniques based on Baermann funnels or Cobb sieving. Seinhorst (1962) concurred, since 50 or 100 ml centrifuge tubes excessively limit the size of the soil sample which can be extracted.

Refining the Caveness and Jensen technique, Gibbins and Grandison (1967) layered 10% over 25% sucrose (weight/volume). Paratylenchus in crude suspension, recovered by the Caveness and Jensen method, was layered above the 25% solution in a centrifuge tube, and the 10% solution was layered over the suspension. Tubes were centrifuged at 2050 G for 30 minutes, further separating the nematodes debris. Harrison and Green (1976) found

that soaking soil in water even briefly before extraction improves recovery by the Caveness and Jensen method.

Centrifugal flotation can be adjusted to fit different soil types. Minderman (1956), using 1.25 sp. gr. magnesium sulfate, recentrifuged not only the resuspended initial pellet but also the initial supernatant when working with clay soils. For sandy soils, however, the initial supernatant was discarded; only the resuspended pellet was centrifuged.

**Wet Sieving Before Centrifugation.** In some variants of the centrifugal flotation method, soil is wet sieved prior to centrifugal flotation. Jenkins (1964) directed a stream of water to wash soil through a 20 mesh sieve into a 10 quart pail. When half full, the pail contents were stirred, left to settle for 30 seconds, and decanted into a 50 or 60 u sieve. After residues were rinsed from the sieves into a beaker, the pail was filled and stirred again, the decanting repeated, and the residues again washed rinsed into the same beaker.

Dunn (1971) compared the efficiency of both sieving-centrifugation and direct centrifugation to Seinhorst elutriation. In the direct method, 50 cc soil was shaken vigorously with 200 ml water in a 250 ml centrifuge bottle and then centrifuged. The supernatant was decanted and the pellet was resuspended in sucrose and centrifuged

again. In the sieving method, 250 cc soil was wet sieved and decanted through a 325 mesh (45 u) sieve before centrifuge processing. Direct centrifugation consistently recovered more Criconemella sp. and about equal numbers of Pratylenchus penetrans and Xiphinema americanum as the other two methods.

Dickerson (1977) evaluated the direct centrifugal flotation procedure by comparing it with sieving-centrifugal flotation and sieving followed by Baermann funnel methods (a modified Christie and Perry method). The direct method was low in error, required little operator skill, and gave the most consistent results, but the samples contained more debris.

**Efficiency of Density Centrifugation Methods:** Using a wet sieving-density centrifugation method, Weber and Williams (1968) evaluated extraction efficiency for Hoplolaimus sp., Helicotylenchus sp., and Criconemoides sp. All discarded water as well as the supernatant comprising the original sample was evaluated for nematode recovery by passage through 45 and 38 u sieves. The 45 u sieve recovered 35.4% of Hoplolaimus, 47.7% of Helicotylenchus, and 41.5% of Criconemoides from the supernatant. Additional washes and sugar centrifugations of sediment remaining after the initial wet sieving increased recovery total to 39.3% of Hoplolaimus, 53.3% of

Helicotylenchus, and 48.9% of Criconemoides on the 325 mesh sieve. Passing through the 45 u to the 38 u sieve were an additional 0.8% of Hoplolaimus, 6.3% of Helicotylenchus, and 5.9% of Criconemoides. In the water after the first centrifugation in water were 23.9% of Hoplolaimus, 15.7% of Helicotylenchus, and 2.2% of Criconemoides. An additional 1.5% of Helicotylenchus and 1.4% of Criconemoides passed through the 38 u sieve. Recovery of Criconemoides from 600 cc soil samples was less efficient than from 150 cc samples, while Hoplolaimus and Helicotylenchus recoveries were unaffected by soil sample size.

Caveness and Jensen (1955) and Seinhorst (1962) credit the centrifugation-flotation extraction method with being the only option for separating eggs from soil. However, Barker and Nusbaum (1971) state that no satisfactory method is available for detection and identification of eggs, which may be the principal fraction present after soil treatments with nematicides or at certain seasonal periods.

#### **Adjusting Density Centrifugation for Criconematids:**

The Criconematids have traditionally been difficult to extract. Lawrence and Zehr (1978) noted that density centrifugation extraction from areas known to have high Macroposthonia populations consistently recovered very low

populations from samples collected after prolonged dry weather. They modified Jenkin's centrifugal-flotation procedure to improve extraction of this nematode from dry soils by mixing samples with water and mixed in a Waring Blendor just prior to extraction. Sucrose concentrations of 908, 1,362, and 1,816 g/l of solution were compared to the 454 g/l of solution recommended by Jenkins. In trials comparing differing percentages of moisture for from one to seven days, the best results among three soil types were obtained when soil was moistened to 16 - 24% at least 24 hours before extraction. With certain soil types, increasing the sucrose concentration was more effective than soil moistening.

**Flotation Sieving with Flocculants:** Byrd et al. (1966) described a rapid flotation-sieving extraction procedure suitable for most nematodes which used flocculating agents instead of a centrifuge to separate nematodes from soil particles in a sucrose solution. Separan 2610 (Dow Chemical Company, Midland, Michigan) as a flocculating agent was unaffected by soil type and pH. Ferric chloride was also satisfactory, but the rate of recovery varied greatly.

In this method, 50 cc of soil were placed in a 600 ml beaker with sufficient 1.0 M sucrose solution containing different concentrations of Separin (1.57 to 25 ppm) or



ferric chloride (9.6 to 144 ppm) to bring the volume to 350 ml. After motorized stirring, the solution was allowed to settle for two to five minutes. The suspension was then decanted through stacked 355 and 45 u sieves, and the residue from the 355 mesh sieve was rinsed onto the 45 u sieve. The material on this sieve was rinsed into a beaker, allowed to settle, and again poured through the 325 mesh sieve. Sieve contents were then rinsed into a counting dish. The rate and duration of stirring was critical, since stirring rate needed to be low for the colloids to remain in suspension. This method required about one to three min per sample and compared favorably with other standard procedures.

When compared to Baermann funnels and Jenkins' (1964) centrifugal flotation method, sugar-flotation-sieving effectively recovered most ectoparasites including Xiphinema sp., Trichodorus sp., spiral nematodes, and Tylenchorhynchus (Barker et al., 1969). However, comparative recovery of ring nematodes and other inactive forms was poor (Byrd et al., 1966; Hooper 1986a). Ring nematodes' coarse annulations caused specimens to be carried out of suspension with flocculating debris (Barker and Nusbaum 1971) Barker et al. (1969) indicated that Ayala et al. (1963) found the sugar-flotation-sieving technique superior to three other methods. One exception,

however, was Pratylenchus zeae, which was recovered in much higher numbers from Baermann funnels (Barker et al., 1969). During winter, when nematodes are less motile, sugar-flotation-sieving yields much higher numbers of most nematodes than Baermann funnel extraction (Barker and Nusbaum, 1971). These authors speculated, however, that the opposite may occur during summer or fall when most nematodes are very active.

**Comparisons of Extraction Methods.** Relative efficiency data compiled by Barker (1985) indicated that Baermann funnels, extracting 35-45%, are the most efficient for Pratylenchus. Centrifugal flotation and wet sieving/Baermann funnel extractions yielded 17-29% and 22-36%, respectively. For Criconemella, however, Baermann funnels were the least efficient, extracting 1-15%. Elutriation and density centrifugation yielded 70-79%, while density centrifugation alone yielded 25-62%. (The latter two methods are comparable to density centrifugation with and without prior wet sieving.)

In comparing cottonwool-modified Baermann funnels with Cobb sieving, Townshend (1963a) found that Pratylenchus penetrans, an active nematode, was extracted best by the Baermann modification. Xiphinema americanum and Criconemoides curvatum, sluggish nematodes, were extracted best by Cobb sieving. This supports the results

of Oostenbrink (1960), in which more Tylenchorhyncus and Rotylenchus were extracted by flotation than by cottonwool. Direct centrifugation consistently recovered more Criconemoides and about equal numbers of Pratylenchus and Xiphinema than did sieving-centrifugation or elutriation (Dunn, 1971). Direct centrifugation was the least laborious and time-consuming of these three methods and was recommended when attempting recovery of many different kinds of nematodes from soil.

In comparing extractions of many genera from different soil types by sedimentation, motility, sieving, and centrifugation methods, Harrison and Green (1976) found that centrifuging was the most versatile and the best for clay soils. Baermann funnels were generally poor, especially for sandy soils and for Tylenchorhyncus. Rotylenchus was the only genus extracted equally well by all techniques. Centrifugal extraction was selected as the routine method for surveys, since it is effective on all soil types, less likely to be subjected to variation, and, although it is poor for Longidorus, extracted a wider spectrum of nematodes than other methods.

Barker et al. (1969) investigated seasonal population dynamics measured by Baermann funnels, centrifugal flotation as modified by Jenkins (1964), and sucrose flotation sieving. Population curves of six different

nematodes differed in maxima and minima according to extraction method and season. Baermann funnels gave the highest recoveries of Meloidogyne spp. and Pratylenchus zeae during summer and fall, but centrifugal flotation and sucrose flotation sieving yielded higher numbers of these nematodes in winter and spring. Centrifugal flotation was the only effective method for recovering Criconemoides ornatum. Centrifugal flotation consistently gave higher yields of Helicotylenchus dihystera throughout the year than the other two methods (Barker, Nusbaum, and Nelson, 1969). Centrifugation was shown to be more efficient than dynamic methods during the coolest times of year (McSorley and Parrado, 1982). Funnels were ineffective for Macroposthonia sp. and Hemicriconemoides mangiferae.

In comparing the Christie and Perry method to centrifugal flotation, Bravo (1977) found that the Christie and Perry method produced more active Xiphinema mediterraneum than did centrifugal flotation. Yields from the Christie and Perry method were similar to centrifugal flotation to which two or more additional centrifugations with zinc sulfate had been added or to five centrifugations with sucrose solution. The Christie and Perry method was reported to be easier and its efficiency higher for X. mediterraneum.

Baermann funnels and centrifugal flotation were compared for extracting Ditylenchus dipsaci from organic soil. Centrifugal flotation gave the highest yields and almost complete separation from soil particles (Lewis, 1960).

In comparing Baermann funnels with sucrose flotation from clay and sand, Kimpinski and Welch (1971) found that sucrose flotation recovered 42.1% of nematodes from clay and 37.5% from sand. Baermann funnels recovered 25.8% from clay and 25.3% from sand.

Four density solutions used in centrifugal flotation were compared with elutriation/cotton wool separation by Decraemer, Coolen, and Hendrickx (1979). Centrifugal-flotation was significantly better than elutriation, but no significant differences were detected between numbers extracted by the four solutions.

Vigliierchio and Schmitt (1983) compared five commonly used extraction methods: density flotation, wet sieving/sucrose centrifugation, wet sieving and Baermann extraction, sieving of soil followed by Baermann extraction under mist, and soil placed directly on funnels under mist. Extraction efficiency was observed to be dependent upon method, soil type, and nematode species. In all soils, density flotation and wet sieving/sucrose centrifugation were the most effective for Criconemella

xenoplax, yielding 22% and 26%, respectively, from clay soils; 48.1% and 48.3% from sandy soils, and 34.9% and 38.9% from loamy sand. In contrast, density flotation and wet sieving/sucrose centrifugation were among the least effective methods for Pratylenchus vulnus except in loamy sand, in which density flotation was the most effective, extracting 37.3%. Sieving followed by Baermann extraction was the most effective in both sandy loam and clay loam, yielding 45.4% and 42.2%, respectively. Sieving of soil followed by Baermann funnel extraction was the most efficient for extraction of Meloidogyne incognita from sandy loam and loamy sand, yielding 40.2 and 44.3%, respectively. No one method was significantly better than others for extraction of this species from clay loam. Soil sieving followed by Baermann funnel extraction was the most efficient for extracting Xiphinema index from clay loam and loamy sand, yielding 30.2 and 30.7%, respectively. Soil sieving followed by Baermann funnel extraction of sievings in a mist chamber was the most effective for extraction of X. index from sandy loam, yielding 32.5 per cent.

Viglierchio and Schmitt (1983) warned that conversion factors in excess of 2 to arrive at an estimate of initial population levels in a sample are highly risky for a quantitative method and are untrustworthy for disease

threshold determinations.

## EXTRACTION OF NEMATODES FROM ROOTS

Although an entire population of ectoparasites may be estimated through soil extraction, complete assessment of an endoparasitic population requires extraction of nematodes from plant tissue as well as soil. Nematodes may be removed from plant tissue by mechanical or dynamic methods. Mechanical methods rely on external force or manipulation to separate nematodes from tissue and include mechanical, chemical, and enzymatic maceration. Dynamic methods rely on nematode motility and include Baermann funnels, incubation, and mist chamber extraction.

**MECHANICAL METHODS: Mechanical Maceration and Sieving.** Mechanical maceration can be used prior to either mechanical or dynamic extraction methods (Thorne, 1961). An early use of maceration of the substrate was to remove Ascaris lumbricoides from intestinal tissue and Trichinella spiralis from muscles. Maceration using a blender has worked well for recovery of endoparasites from plant tissues (Murphy, 1962).

To extract Pratylenchus, Radopholus, and other endoparasites, Thorne (1961) suggested cutting roots into 10-20 mm segments and processing them in a Waring Blendor for 20 to 60 seconds or until reduced to small fragments.



Residues were then placed on Baermann funnels for 12-24 hours or washed through sieves and examined immediately. Escobar and Rodriguez-Kabana (1980) compared simple sieving of macerated banana root samples to a flotation-sieving technique for extraction of Radopholus similis and found that results were comparable.

Maceration and sieving techniques are an efficient method for extracting endoparasites, especially if nematodes are needed immediately. Inactive or damaged nematodes can be recovered, which is impossible using methods relying on nematode motility (Loos and Loos, 1960; Hooper, 1986). Macerating roots mechanically combined with sieving and Baermann funnels gave yields similar to mist chamber extractions, in which tissue was placed on screens over funnels draining into test tubes and misted at frequent intervals (Barker and Nusbaum, 1971). Adding hydrogen peroxide to the incubation solution improved oxidation and recovery (Hooper, 1986).

Maceration may damage more delicate nematode species, and determination of the number alive before maceration is difficult (Murphy, 1962). Minderman (1956) reported that maceration by Waring Blendor halved about 30% of the nematodes in oak forest litter. Macerating tissues in a blender is useful in some cases, but it is not recommended

due to the release of toxic substances by some plants (Barker, 1985).

**Chemical or Enzymatic Masceration.** Recovery of nematodes from infected roots by chemical and enzymatic masceration was investigated by Dropkin et al. (1960). Roots infected with Meloidogyne sp. were subjected to three commercial enzyme preparations derived from fungal cultures and fluid from Erwinia carotovora-infected potato slices. Chemical treatments were hydrochloric acid, periodic acid and Jeffrey's macerating fluid. After exposure times varying from three to eight hours, enzyme-treated tissues became translucent except for the xylem, which was unchanged. This translucence was more pronounced in the infected potato fluid than in commercial preparations. Intact nematodes were released during enzymatic masceration, and all stages were present in good condition. Further mechanical masceration released additional nematodes. Eggs recovered by this method hatched into juveniles infective to tomato plants.

All chemicals used for masceration softened plant tissues. However, nematode cuticles were broken in hydrochloric acid. Intact dead nematodes were recovered by sieving after treatment with periodic acid or Jeffrey's solution. The latter solution stained giant cells brilliant yellow and thus more noticeable.

**DYNAMIC METHODS: Baermann Funnels.** Baermann funnel extraction of tissue is similar to funnel extraction of soil. Adding a trace of wetting agent and a few ml of 0.15% methyl p-hydroxybenzoate solution to the funnel liquid inhibits bacterial and fungal growth which can interfere with counting and hasten nematode decomposition (Hooper, 1986). Baermann funnels have limited use in tissue extractions, since yield decreases sharply when the sample size exceeds 3 to 4 grams of roots (Chapman, 1957). Minderman (1956) devised an aerated Baermann system in which 300 mg of oak forest litter was placed on a screen raised two centimeters from the bottom of a beaker. The beaker was filled half full of water and shaken periodically for 24 hours. More nematodes were extracted from samples of oak forest litter by this process than were extracted by the Baermann Funnel method.

**Incubation.** Incubation refers to the maintenance of tissue containing migratory endoparasitic nematodes in conditions conducive to the emergence of the nematodes. The mechanisms involved in recovery of migratory endoparasitic nematodes from incubated roots are not well understood (Bird, 1971). They may be attracted to the incubation solution, or stimulated to move away from their normal feeding sites, or both.

Young (1954) described an incubation method for collecting migratory endoparasitic nematodes. Avocado roots containing Radopholus similis were washed and placed in Mason jars in moist condition and incubated at room temperature. Within a few hours, nematodes started accumulating in the small amount of water which had drained from the roots. As the water in the jar was exhausted by sampling, it was replaced by occasional spraying with a wash bottle, moistening the roots and attracting more nematodes. Over 3000 R. similis were isolated from a pint of loosely packed avocado roots over two weeks. Young placed one half of each of 14 samples in Baermann funnels and the other half in jars. Total yield was 805 from the funnels and 3,761 from jars.

When incubating roots in shaken flasks, Bird (1971) found that, at all intervals during the first three weeks after roots had been removed from the soil, all populations of Pratylenchus brachyurus consisted of approximately 25% J2, 44% J3, and 31% female. Per cent recovery depended on sieving techniques to concentrate nematodes and separate them from debris. A single 325 mesh sieve was not adequate for recovery of most of the second, third, and fourth stage juveniles, but 84% of the females were recovered on this size sieve (Bird, 1971).

Considerations of mesh size used in root extractions are the same as for soil extractions, as discussed earlier.

**Controlling Microbial Contaminants.** Fungal and bacterial contaminants need to be controlled in incubation techniques. In quiet (non-shaken) flasks, nematode recovery increased as temperature increased to 35C, but the flasks were soon contaminated with fungus and bacteria (Chapman, 1957). Growth of microbial contaminants limited the standard incubation period using the Young (1954) moisture incubation method to three to five days. A longer incubation period yielded higher numbers, but after seven days, all nematodes were dead. Immersing the sample in water caused even more rapid deterioration of nematodes (West, 1957).

Townshend (1962) reduced microflora while extracting nematodes from roots by adding .0004% ethoxyethyl mercury chloride/.1% dihydrostreptomycin sulfate solution to the solution bubbling through a modified Andus soil perfusion apparatus. While investigating other chemicals, either as anti-microbial agents or nematode stimulants, Bird (1971) found that the rate of recovery of Pratylenchus brachyurus from cotton roots was enhanced when tissue was incubated in solutions containing 10 ppm ethoxethyl mercuric chloride, 50 ppm dihydrostreptomycin sulfate, 50, 100, or 1000 ppm diisobutylphenoxethyl dimethyl benzyl ammonium

chloride, or a mixture of these compounds, or in 10 or 100 ppm zinc sulfate, zinc chloride, or magnesium chloride (Bird, 1971). Incubation in 1 or 1000 ppm zinc chloride or magnesium chloride had no influence on this phenomenon, whereas 10,000 ppm zinc chloride, zinc sulfate, or magnesium chloride retarded the rate of recovery.

**Oxygen Supply.** Inadequate oxygen supply can limit yields from incubation extractions and has been addressed by several innovations. Chapman (1957) compared root sample treatments of shaking in flasks of water, of bubbling air through flasks, and of spraying roots with mist to extract Pratylenchus scribneri. Bubbling increased yield 86% over non-bubbled flasks for heavier root samples and 26% for lighter root samples. Sprayed samples yielded 179% more nematodes than non-bubbled water incubated samples. Pratylenchus vulnus was extracted by bubbling air through water in 21 cylinders in which root samples were immersed (Osborne and Jenkins, 1963). Nematodes were collected by pouring the resulting suspension through a 45 u sieve, which was then rinsed several times to remove contaminating organisms.

Consistently more Radopholus similis were obtained from roots incubated in oxygen permeable polyethylene bags than in glass jars for periods of up to nine days at 23.9 C (Tarjan, 1960). Incubation in plastic bags was also

superior to Baermann funnel extractions. Further tests combining different incubation temperatures with hydrogen peroxide and water as incubation solutions were performed. Incubation in 1-3% hydrogen peroxide at 20.6-21.6 C produced the highest yields (Tarjan, 1967). Similar experiments with Tylenchulus semipenetrans juveniles and males produced the greatest yields using 10 ml of 3% hydrogen peroxide at 21.1 C.

Gower and Edmunds (1973) obtained more Radopholus similis and Helicotylenchus multicinctus from incubation of macerated banana roots in dilute hydrogen peroxide at 27-31 C for two days than from roots chopped into 2-4 cm lengths incubated in tap water for two days. Maceration until cortical tissue just separated from vascular tissue was optimal for nematode extraction. The optimum hydrogen peroxide concentration was 10 ml of 30% solution in one liter of tap water. Two-day hydrogen peroxide incubation yielded nematode numbers similar to those from more laborious direct extraction techniques.

**Incubation Temperature.** The effect of heat on the emergence of Pratylenchus penetrans during incubation was investigated by Dolliver (1959). Emergence rate increased with temperature up to 24 C but was inhibited by 10 minute 24 C root treatments. Longer treatments of 43 C caused severe but not permanent inhibition of emergence. A

dilute salt solution also inhibited P. penetrans emergence.

**Limitations of Root Incubation.** Root incubation has limitations, however. Chapman (1957) found that incubation has limited use in tissue extractions, since yield decreased sharply when the sample size exceeded 1 to 2 g in Petri plate incubations. Similarly, Gowen and Edmunds (1973) obtained greater yields in hydrogen peroxide aerated incubation from 20 g than from 40 g samples. If the root mass being extracted is too thick, nematodes may fail to pass through due to starvation, exhaustion, or both. Barker (1985) found incubation, even enhanced by an airstream, of limited use and less efficient than mist extraction.

**Mist Extraction.** In the Seinhorst "mistifier", a fine mist of water is sprayed continuously over infested tissue (Seinhorst, 1950, in Hooper, 1986). Active nematodes that emerge are collected in the water below the sample. Because of better oxygenation, and possibly because toxic compounds are washed away, nematodes recovered by this method are often more active than those derived by other methods. Mist extraction is effective for bulbs, leaves, stems, seeds, roots, litter, and mushroom compost. Because Rhadinaphelenchus spp. swim and



are lost in the overflow, this method is ineffective for this genus.

Barker (1985) considered mist extraction the best method for plant tissue but took a "relatively long" three days, and resulting data were more qualitative than quantitative. Since nematodes continued to develop in tissues, and many were recovered after two or more weeks of extraction, a three day extraction recovered only a fraction of the total population.

Wall and Chapman (1967) investigated prolonged mist extraction of Pratylenchus penetrans from different sample sizes. More nematodes per gram were collected from samples of 0.9 than from 1.8 or 3.6 g. Nematodes continued to emerge for up to 19 days. Additional experiments established that the inverse yield to sample size relationship was not due to trapping of nematodes between roots, and the real cause was not determined.

Chapman (1957) found that Pratylenchus sp. extracted by incubation emerged from roots at an irregular rate and that there was no point in time at which a reliable percentage of the total could be obtained. No form of aeration improved this trend, and after 29 days of incubation, Pratylenchus penetrans was still emerging in appreciable numbers. He recommended that samples must be

kept until they showed definite signs of being exhausted of nematodes.

Removal of senescent tissue before extraction resulted in reduced Ditylenchus dipsaci numbers recovered, but contamination by non-parasitic microbial feeding nematodes was reduced from about 10% to 0.3% or less (Gibbins and Grandison, 1967).

## CONCLUSION

A survey of soil extraction methods reveals not only some strengths and weaknesses of each but also an almost infinite source of possible variations. An overview of comparisons of these methods provides direction and recommendations, although differing in some cases, about methods appropriate for a given situation. In some cases, methods can be adjusted to greater efficiency for target species, while in other cases either adjustments are not possible or target species are physically diverse enough to require radically different extraction methods.

The different methods of extracting plant-parasitic nematodes from tissue have alternative advantages and disadvantages. While mist chamber and incubation extractions may take a relatively long time and facilitate anaerobic conditions and microbial growth, faster mechanical techniques may fragment nematodes. Chemical and enzymatic maceration techniques recover whole nematodes, but appearance and activity may be altered. Realistic assessment of entire populations is often not possible within time constraints of diagnostic laboratories. Direct comparisons of yields from different extraction techniques are often not possible. Thus,

experiments comparing results from different extraction.  
methods are needed.

**EFFICIENCY OF EXTRACTING PRATYLENCHUS PENETRANS,  
PARATYLENCHUS SP., CRICONEMELLA XENOPLAX,  
AND MELOIDOGYNE CHITWOODI**

Study of nematode populations in soils is dependent on 1) thorough and representative soil sampling, 2) reliable extraction procedures, and 3) accurate counting of samples. Effectiveness of soil sampling and nematode counting are largely controlled by the skill, diligence, and expertise of the operator. However, extraction procedures are affected by factors which the researcher may not be aware of or able to control. Several factors including soil texture, pH, temperature, storage conditions, extraction method, equipment used, and nematode species can affect the efficiency of soil nematode extraction. There are advantages and disadvantages to any extraction method, and different methods may be appropriate for different situations. The extraction method used is not as critical as is the ability to correct for efficiency. If results are properly corrected for efficiency, they should be the same regardless of the method used. Determining extraction efficiency is difficult and time consuming and should be done for each nematode species and each soil type. Thus,

few laboratories determine or report extraction efficiency on a routine basis.

In the following studies, various modifications were made to routine extraction methods to determine the efficiency of various methods and whether the efficiency could be improved.

## MATERIALS AND METHODS

**Wet Sieving/Sucrose Centrifugation (WS/SC).** Soil samples (100 g) in small beakers were presoaked for 10 minutes in 100 ml tapwater before extraction. The soil was then rinsed from the beaker and washed through an 1 mm (18 mesh) sieve into a bucket. The bucket receiving the soil suspension was filled with five liters of cool tap water, stirred vigorously, and allowed to settle for two minutes. Water from the bucket was then poured slowly through a 38  $\mu$  (400 mesh) sieve held at a 45 degree angle. Nematodes and soil left on the sieve were rinsed into 100 ml polycarbonate centrifuge tubes which were balanced and centrifuged at 3000 rpm for three min. Half of the supernatant was decanted without disturbing the soil pellet at the bottom of the tube, and debris adhering to the walls of the tube was removed. The tube was refilled with sucrose solution (900 g sucrose/liter of solution) and the pellet resuspended by vigorous shaking. The tube was centrifuged again at 3000 rpm for three min. The sucrose solution was decanted onto a 38 micron sieve, which was partially immersed in water at a 45 degree angle so that nematodes and sucrose dispersed on the water before settling onto the sieve. The sieveings were rinsed

into a conical centrifuge tube and allowed to settle for at least 12 hours. Excess water was decanted, and the nematodes and remaining water were poured into a counting dish and counted. Any variations in this method are explained for individual experiments below.

**Baermann Funnel Method (BF).** Soil samples (100 g) were placed on Rapid-Flo milk filters (Filter Fabrics, Inc., 814 E. Jefferson Street, Goshin, IN 46526) which were supported by a piece of plastic window screen glued to a 4-cm-diameter 2-cm high ring of PVC pipe. The screen/filter/soil complex was placed in a 12.4 cm diameter plastic funnel which had been filled with tap water to within one cm of the rim. A piece of latex tubing attached to the funnel stem was clamped at the bottom to retain water. Samples remained in the funnels for five days, and the water level in each funnel was checked daily. At the end of five days, about 150 ml of water was drained from the bottom of the funnel, and poured through a 25  $\mu$  sieve to concentrate the nematodes. Nematodes collected on the sieve were rinsed into a counting dish and counted. Any variations in this method are explained for individual experiments below.

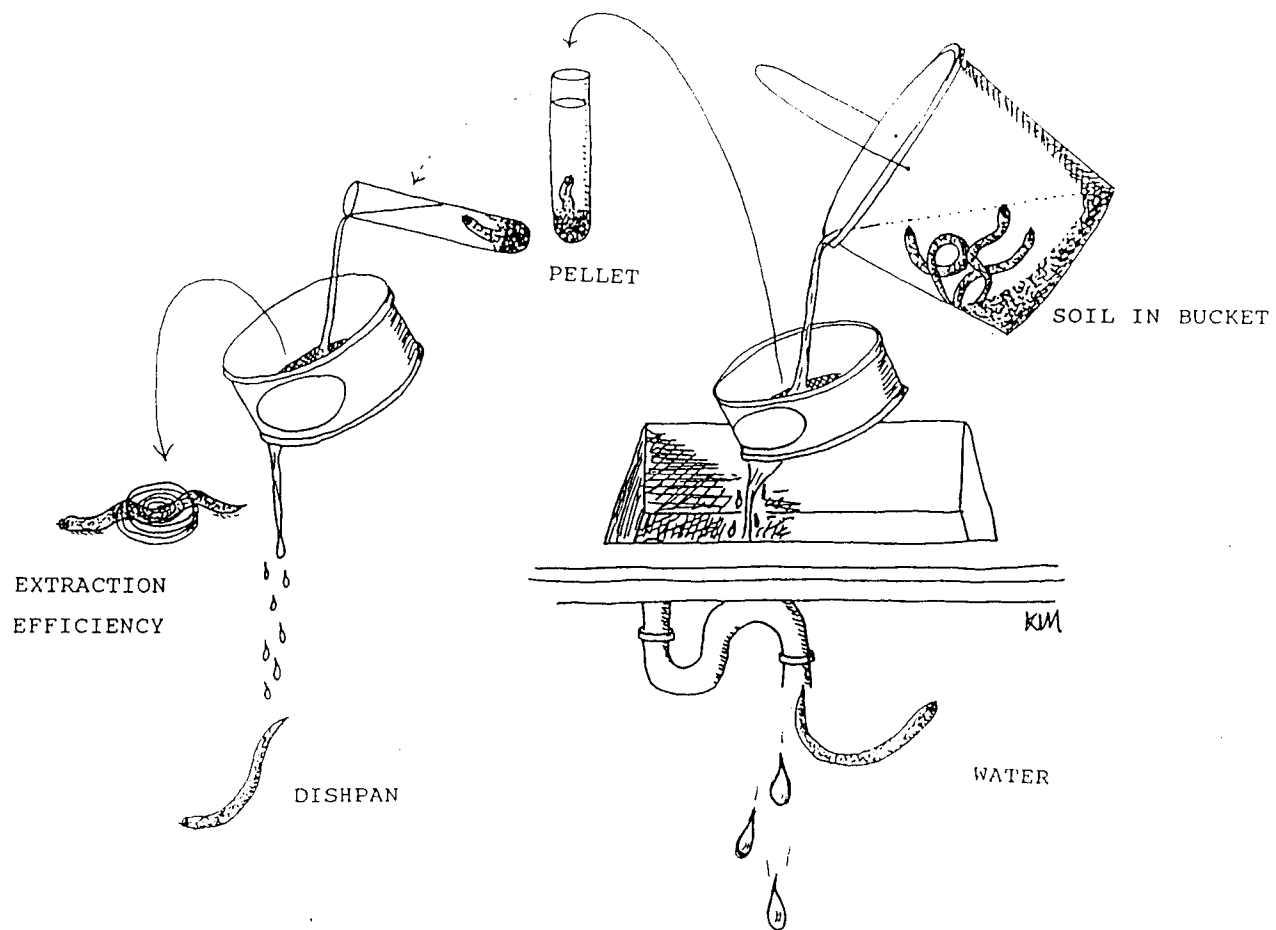
The soil from which nematodes were extracted in all experiments except experiment 5 was a silt loam with 11% clay, 58% silt, 31% sand, and <0.1% coarse particles.



### Experiment 1: Determination of WS/SC Extraction

**Efficiency:** The objectives of this experiment were 1) to determine the efficiency of WS/SC for P. penetrans, Paratylenchus sp., and C. xenoplax and 2) to determine where nematodes of these three species were not being recovered in this extraction process. Soil collected from a Willamette Valley peppermint field near Monroe, Oregon on October 9, 1989 was sieved through a 5 mm mesh sieve and thoroughly mixed. Ten 100 g samples were treated as follows: Soil was rinsed through a 1 mm mesh sieve into a bucket, and water volume increased to 7.5 l. The suspension in the bucket was stirred, allowed to settle for two minutes, and poured through a 38 u (400 mesh) sieve while catching the water passing through the sieve in another bucket below. Soil and nematodes remaining on the 38 u mesh sieve were rinsed into a centrifuge tube and will be referred to as the "standard sample". The water caught after sieving was poured through a 25 u (500 mesh) sieve, catching the water in another bucket below. This water was poured through the 38 u mesh sieve again, and the sieve contents from both pourings of water were rinsed into a second tube labeled "water". The soil in the bottom of the original bucket was resuspended in clean tapwater and immediately poured through a 38 u mesh sieve without being allowed to settle. Sieve contents were

rinsed into a third tube labeled "bucket soil". This process was repeated, and the sieve contents were again rinsed into the "soil" tube. The "water" sample, "bucket soil" sample, and soil from the "standard sample" was processed by sucrose centrifugation as described above. The diluted sucrose solution remaining in the dishpan after the sucrose solution from the standard sample was poured through the 25 u mesh sieve was poured slowly through the 25 u mesh sieve and the sieve contents were rinsed into a bottle labeled, "dishpan". The soil pellet in the centrifuge tube was resuspended in sucrose solution, diluted with equal parts water, centrifuged, and poured through the 25 u mesh sieve. The sieve contents were rinsed into a bottle labeled "resuspended pellet I". This step was repeated and the sieve contents rinsed into a second bottle labeled "resuspended pellet II". P. penetrans, Paratylenchus sp., and C. xenoplax in each fraction were counted and summed to determine the total number of nematodes that had been in the initial soil sample. The WS/SC extraction efficiency determination process is illustrated in Figure 1.



**FIGURE 1. WET SIEVING/SUCROSE CENTRIFUGATION  
EXTRACTION EFFICIENCY DETERMINATION**

The percent of total nematodes that were recovered from each fraction of the extraction procedure, as represented by labeled bottles, was determined, and percent extraction efficiency for each species was then calculated. The mean percentage of each nematode species recovered from each extraction fraction was compared by ANOVA. Numbers from the soil in the bucket and both pellets were combined as a measure of nematodes settling during the process, and percentages from the water and dishpan fractions were combined as a measure of nematodes passing through the 25  $\mu$  sieve. Averages of these percentages were compared between species by ANOVA. All percentage data were arcsine-square root transformed before analysis.

**Experiment 2: BF Extraction Efficiency and Daily Recovery Rate.** The objectives of this experiment were 1) to compare the Baermann funnel method with the wet sieving/sucrose centrifugation method for recovery of P. penetrans, Paratylenchus sp., and C. xenoplax from Willamette Valley field soil; 2) to determine the number of each species left in the soil after the Baermann funnel extraction; and 3) to determine nematodes recovered from Baermann funnels on a daily basis.

Soil collected from a peppermint field near Monroe, Oregon was sieved through a 5 mm mesh sieve and thoroughly mixed. Ten 100 g samples each were extracted by one of the following procedures. Wet sieving/sucrose centrifugation was performed as described above. Baermann funnel extractions were performed as described above with the following modifications: Samples were left in water for six days, and each funnel was drained and nematodes counted daily. After the sixth day, the soil from each funnel was removed for WS/SC extraction of remaining

nematodes. P. penetrans, Paratylenchus sp., and C. xenoplax recovered from BF, WS/SC of the BF samples, and WS/SC alone were counted using a dissecting microscope. Numbers of nematodes from each sample recovered by WS/SC were corrected for efficiency according to the results from experiment 1. Student's t-test was used to compare log transformed data from the BF-WS/SC combination with that from WS/SC alone. The total recovered from BF and subsequent WS/SC of Baermann samples, and the percentage of that total recovered by BF alone and by WS/SC alone were calculated. Percentage data were arcsine-square root transformed before analysis. Daily totals from BF extractions were added cumulatively, averaged, and graphed.

**Experiment 3: Effect of Short Term Storage Time on WS/SC Nematode Recovery.** The objective of this experiment was to determine the effects of short term cold storage on recovery of nematodes by WS/SC extraction. Soil collected November 6, 1989 was screened through a 5 mm sieve and mixed thoroughly. That day, ten 100 g samples were processed by WS/SC as described above, and the remaining soil was stored at 3.3 C (38 F). On November 7 and November 8, ten more samples were extracted by the same procedure. Log transformed means of P. penetrans,

Paratylenchus sp., and C. xenoplax for each day's extraction were analyzed by ANOVA.

**Experiment 4: Effect of Long Term Cold Storage on WS/SC and BF Nematode Recovery.** The objectives of this experiment were 1) to compare the recovery of P. penetrans, Paratylenchus sp., and C. xenoplax from BF and WS/SC extraction procedures and 2) to determine any differences in recovery of the three nematodes by the two methods on four dates during a two month storage period.

Peppermint field soil was collected on September 1, 1988, screened through 5 mm sieve, and mixed thoroughly. On September 1, September 29, October 12, and November 9, four 100 g samples each were extracted using BF or WS/SC procedures as described above. Between extraction dates, soil was stored in sealed plastic bags at 3.3 C (38 F). P. penetrans, Paratylenchus sp., and C. xenoplax in each sample were counted using a dissecting microscope. ANOVA was performed on log transformed data for each species by date, and by method.

**Experiment 5: Effect of Extraction Method and Date on M. chitwoodi Recovery.** The objectives of this experiment were 1) to compare recovery of Meloidogyne chitwoodi by Baermann funnel extraction with recovery by wet sieving/sucrose centrifugation, 2) to compare efficiency of two types of filter material for BF

extraction of M. chitwoodi, 3) to examine daily recovery of M. chitwoodi from Baermann funnels, and 4) to compare differences in recovery of M. chitwoodi by the same methods after 22 days of storage at 3.3 C (38 F).

On February 24, 1988, ten 100 g samples of composited soil from several greenhouse pot cultures of M. chitwoodi on wheat were processed by BF extractions as described above. An additional ten 100 g samples were extracted using Kimwipes (Kimberly-Clark Corporation, Roswell, Georgia 30076) as the filter material rather than milk filters. Funnels were drained daily for 5 days, and nematodes recovered were counted daily and added cumulatively. Ten 200 g samples were extracted using WS/SC as described above. On March 18, 1988, five 100 g samples were extracted by each BF treatment and five 200 g samples by WS/SC. Nematodes recovered were counted using a dissecting microscope, and densities from WS/SC-processed samples were divided by two. ANOVA was performed on log transformed data by method and by date. Daily BF counts from each funnel were added cumulatively and averaged for each date to determine the percent of the total population recovered during that time period.

**Experiment 6: Daily Recovery of P. penetrans Recovered from Roots in a Mist Chamber.** The objective of this experiment was to examine the daily recovery of P.



penetrans extracted from mint roots in a mist chamber.

Ten root samples collected from a Willamette Valley peppermint field on May 18, 1989 were trimmed from rhizomes and placed on funnels in a mist chamber for extraction of P. penetrans. For the first 23 consecutive days, extraction tubes were drained daily and nematodes recovered were counted daily. Extraction tubes were drained and nematodes counted every few days between day 23 and day 38. Numbers recovered each day were converted to percentage based on the total recovered from each sample.

## RESULTS

**Experiment 1: Determination of WS/SC Extraction Efficiency.** Distribution of nematodes recovered from the various steps of the WS/SC procedure are summarized in Table 1. Fifty-nine percent of P. penetrans extracted using WS/SC were recovered in the extraction fraction treated as the standard sample. Thirty two percent were recovered from water or sucrose poured through fine mesh sieves, and 8.8% were recovered from three sources of sediment. Of Paratylenchus sp. extracted, 80.2% were recovered in the standard sample, 11.3% from sediment, and 9.3% from water or sucrose poured through fine mesh sieves. Of C. xenoplax extracted, 66.1% was recovered from the standard sample, 16.4% from sediment, and 17.5% from water or sucrose. Percentages recovered from all components of the extraction process except the dishpan differed significantly among species. Sieving losses were much greater than settling losses for P. penetrans, but sieving and settling losses were nearly equal for Paratylenchus sp., and C. xenoplax. Figure 1 illustrates losses in the WS/SC extraction procedure for the three nematode species.

TABLE 1  
WET SIEVING/SUCROSE CENTRIFUGATION  
EXTRACTION EFFICIENCY

| -----PERCENTAGES RECOVERED-----        |                                  |                             |                                 |
|--|----------------------------------|-----------------------------|---------------------------------|
| Extraction Fraction                    | <u>Pratylenchus</u><br>penetrans | <u>Paratylenchus</u><br>sp. | <u>Criconemella</u><br>xenoplax |
| Standard Sample <sup>1</sup>           | 59.1 <sup>2</sup> a <sup>3</sup> | 80.2 b                      | 66.1 a                          |
| Water                                  | 20.6 a                           | 7.0 b                       | 11.8 b                          |
| Soil in Bucket                         | 3.8 a <sup>4</sup>               | 1.7 ab                      | 5.1 b                           |
| Pellet I                               | 4.3 a                            | 6.5 ab                      | 9.3 b                           |
| Pellet II                              | 0.7 a                            | 2.4 b                       | 2.0 b                           |
| Dishpan                                | 1.5 a                            | 2.3 a                       | 5.6 a                           |
| Total Pellet<br>(Pellets I + II)       | 5.0 a                            | 8.9 b                       | 11.3 b                          |
| Total Sediment -<br>(Pellets + Bucket) | 8.8 a                            | 11.3 b                      | 16.4 c                          |
| Sieve Loss<br>(Water + Dishpan)        | 32.0 a                           | 9.3 b                       | 17.5 b                          |

<sup>1</sup>Standard sample = extraction efficiency.

<sup>2</sup>Mean percentages in each extraction fraction (n = 10).

<sup>3</sup>Percentage means followed by the same letters in each row are not significantly different (p = 0.01) using Fisher's Protected LSD procedure.

<sup>4</sup>Percentage means followed by the same letter in this row only are not significantly different (p = 0.05) using Fisher's Protected LSD procedure.

**Experiment 2: BF extraction Efficiency and Daily Recovery Rate.** WS/SC recovered significantly more Paratylenchus sp. and C. xenoplax from peppermint field soil than did BF (Table 2). A low percentage (12.7) of C. xenoplax from the BF-WS/SC combination was recovered by BF, indicating this species' slow migration in BF, whereas higher percentages of P. penetrans (58.2) and Paratylenchus sp. (57.5) were recovered by BF.

TABLE 2

BAERMANN FUNNEL (BF) EXTRACTION EFFICIENCY  
OF PRATYLENCHUS PENETRANS, PARATYLENCHUS SP.,  
AND CRICONEMELLA XENOPLAX  
DETERMINED BY REEXTRACTION BY WET SIEVING/  
SUCROSE CENTRIFUGATION (WS/SC)  
AND COMPARISON OF BF WITH WS/SC.

|   | BF                | WS/SC <sup>1</sup><br>of BF<br>Remains | BF +<br>WS/SC       | WS/SC | BF<br>Efficiency <sup>2</sup> |
|---|-------------------|--|---------------------|-------|-------------------------------|
| <u>Pratylenchus</u><br><u>penetrans</u> | 50.9 <sup>3</sup> | 35.5                                   | 86.4** <sup>4</sup> | 51.4  | 58.2a <sup>5</sup>            |
| <u>Paraty-</u><br><u>lenchus</u> sp.    | 117.7             | 88.4                                   | 204.5**             | 339.9 | 57.5a                         |
| <u>Criconemella</u><br><u>xenoplax</u>  | 8.3               | 58.5                                   | 66.9**              | 281.3 | 12.7b                         |

<sup>1</sup>WS/SC recoveries are corrected using efficiencies determined in Experiment 1.

<sup>2</sup> $[BF/(BF+WS/SC)] \times 100$

<sup>3</sup>Means of extractions of 100 g soil.

<sup>4</sup>Means followed by \*\* are significantly different ( $p = 0.01$ ) from WS/SC means for the same species using Student's t-test.

<sup>5</sup>Means in this column which are followed by the same letter are not significantly different ( $p = 0.01$ ) using Fisher's Protected LSD Procedure.

**Experiment 3: Effect of Short Term Storage Time on Nematode Recovery.** There were no significant differences between average numbers of P. penetrans recovered from extractions over three consecutive days on and following soil sample collection. However, significant differences ( $p = 0.01$ ) were observed in recovery of Paratylenchus sp. and C. xenoplax (Table 3). Both Paratylenchus sp. and C. xenoplax recovery increased on the second extraction day and decreased on the third extraction day so that the number recovered on day 1 and day 3 were equal.

TABLE 3

EFFECT OF SHORT TERM COLD STORAGE<sup>1</sup>  
 ON WET SIEVING/SUCROSE CENTRIFUGATION EXTRACTION  
 OF PRATYLENCHUS PENETRANS, PARATYLENCHUS SP.,  
 AND CRICONEMELLA XENOPLAX

|       | <u>Pratylenchus</u><br><u>penetrans</u> | <u>Paratylenchus</u><br><u>sp.</u> | <u>Criconemella</u><br><u>xenoplax</u> |
|-------|---|------------------------------------|--|
| Day 1 | 56 <sup>2</sup> a <sup>3</sup>          | 445 a                              | 304 a                                  |
| Day 2 | 47 a                                    | 557 b                              | 458 b                                  |
| Day 3 | 45 a                                    | 350 a                              | 278 a                                  |

<sup>1</sup>3.3 C

<sup>2</sup>Nematodes recovered from 100 g soil. n = 10.

<sup>3</sup>Means in columns followed by the same letter are not significantly different (p = 0.01) using Fisher's Protected LSD Procedure.

**Experiment 4: Effects of Long Term Cold Storage on WS/SC and BF Nematode Recovery.** The number of P. penetrans recovered by BF extraction was not significantly different ( $p = 0.05$ ) on any of the four extraction dates during the two month storage period (Table 4). Recovery by WS/SC differed significantly ( $p = 0.05$ ) between extraction dates. However, a decrease on date 2 was followed by increased recovery on dates 3 and 4. Recovery of P. penetrans from BF over all dates was significantly higher than recovery from WS/SC ( $p = 0.01$ ). Paratylenchus sp. recovery did not differ significantly between extraction dates or methods. C. xenoplax recovery differed significantly over two months using either extraction method ( $p = 0.05$  for BF,  $p = 0.01$  for WS/SC). Recovery by BF was extremely low for all dates, while recovery by WS/SC decreased steadily and significantly throughout the storage period. However, mean recovery of C. xenoplax over all dates was significantly higher by WS/SC than mean recovery from BF ( $p = 0.01$ ).



TABLE 4

COMPARISON OF NEMATODE RECOVERY  
FROM BAERMANN FUNNELS AND WET SIEVING/SUCROSE CENTRIFUGATION  
OVER TWO MONTHS OF COLD STORAGE<sup>1</sup>

|         | <u>Pratylenchus</u><br><u>penetrans</u> |       | <u>Paratylenchus</u><br><u>sp.</u> |       | <u>Criconemella</u><br><u>xenoplax</u> |       |
|---------|---|-------|------------------------------------|-------|--|-------|
|         | BF                                      | WS/SC | BF                                 | WS/SC | BF                                     | WS/SC |
| Sept 1  | 95 <sup>2</sup> a <sup>3</sup>          | 12 a  | 505 a                              | 846 a | 5 b <sup>4</sup>                       | 391 a |
| Sept 29 | 62 a                                    | 4 b   | 748 a                              | 607 a | 26 a                                   | 240 b |
| Oct 12  | 75 a                                    | 20 a  | 925 a                              | 571 a | 17 a                                   | 229 b |
| Nov 9   | 64 a                                    | 17 a  | 596 a                              | 535 a | 15 ab                                  | 88 c  |
| Mean    | 74 **                                   | 13    | 693                                | 640   | 16 **                                  | 237   |

<sup>1</sup>3.3 C

<sup>2</sup>Number/100 g soil; n = 4.

<sup>3</sup>Means followed by the same letter in each column are not significantly different (p = 0.05) using Fisher's protected LSD procedure, unless otherwise indicated.

<sup>4</sup>Means in this column only are not significantly different (p = 0.01) using Fischer's protected LSD procedure.

\*\*Means of BF extractions from all 4 dates are significantly different (p = 0.01) from means of wet sieving/sucrose centrifugation extractions from all four dates using Student's t-test (n = 16).

**Experiment 5: Comparison of M. chitwoodi recovery by extraction date and method.** WS/SC recovered significantly more M. chitwoodi than did BF using Kimwipes as filter material in the February 24 extractions, ( $p = 0.01$ ), but there was no significant difference between WS/SC and BF extraction using milk filters (Table 5). On the March 18, 22 days later, WS/SC recovered significantly more than BF extractions using either filter material ( $p = 0.01$ ). No significant difference was observed between dates for WS/SC or BF using Kimwipes, but recovery from BF using milk filters was significantly less ( $p = 0.01$ ) on the second date.

TABLE 5  
 MEAN RECOVERY OF MELOIDOGYNE CHITWOODI  
 BY THREE EXTRACTION PROCEDURES  
 ON SAMPLING DATE  
 AND AFTER 22 DAYS OF COLD STORAGE<sup>1</sup>

|              | February 24 <sup>2</sup>                          | March 18 <sup>3</sup> |
|--------------|---|-----------------------|
| BF:          |   |                       |
| Milk filters | 26,109 <sup>4</sup> a <sup>5</sup> p <sup>6</sup> | 2,963 aq              |
| BF:          |   |                       |
| Kimwipes     | 8,985 bp  | 6,662 ap              |
| WS/SC        | 24,728 ap   | 28,103 bp             |

<sup>1</sup>3.3 C

<sup>2</sup>n = 10

<sup>3</sup>n = 5

<sup>4</sup>J2 recovered/100 g soil

<sup>5</sup>Means followed by the same letter (a,b) in each column are not significantly different (p = 0.05) using Fisher's protected LSD procedure.

<sup>6</sup>Means followed by the same letter (p,q) in each row are not significantly different (p = 0.01) using Student's t-test.

**Baermann Funnel Daily Count:** Daily recovery of nematodes from a 6 day BF in Experiment 2 indicated that 90% of total P. penetrans and Paratylenchus sp. recovered were obtained by the third day. This percentage of C. xenoplax was not recovered until day 5 (Figure 2). Numbers of C. xenoplax recovered by this method are extremely low. In Experiment 5, recovery of Meloidogyne chitwoodi was both slower and lower when using Kimwipes than when using milk filters (Figure 3). Milk filter BF extractions recovered nearly 90% of M. chitwoodi during the first extraction day, while Kimwipe extractions did not recover 90% of the total until day 4.

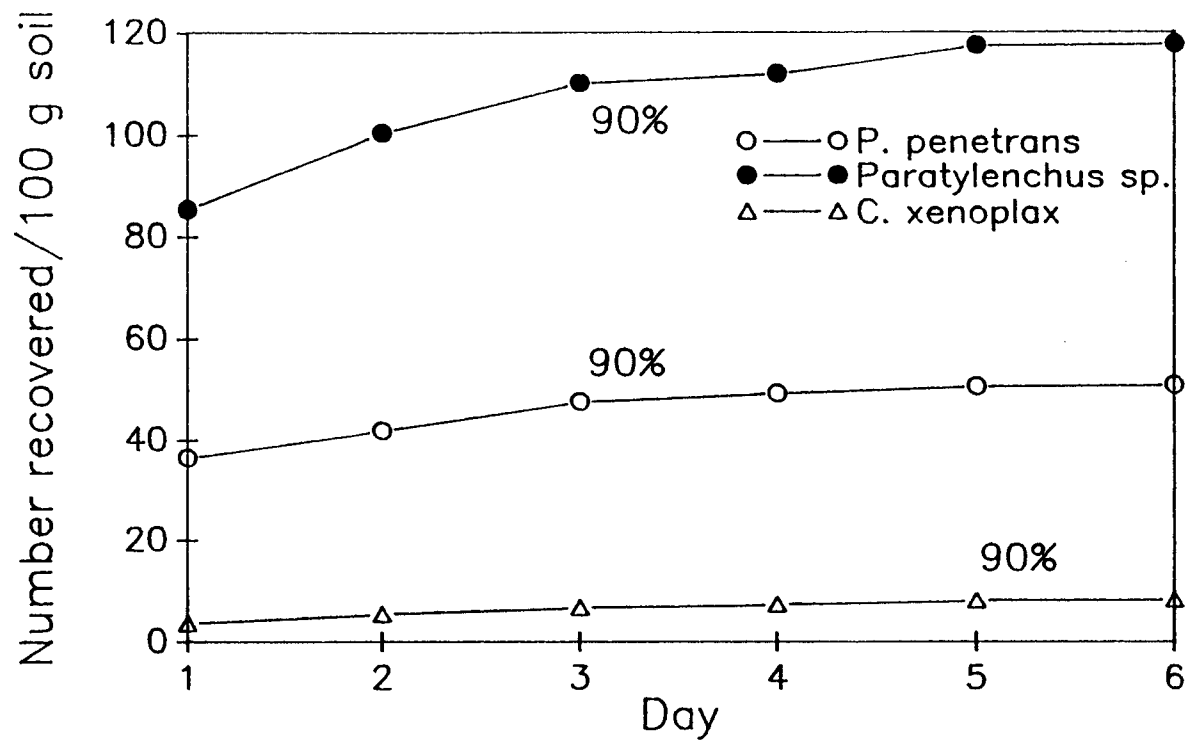


FIGURE 2. CUMULATIVE BAERMANN FUNNEL RECOVERY OF PRATYLENCHUS PENETRANS, PARATYLENCHUS SP., CRICONEMELLA XENOPLAX

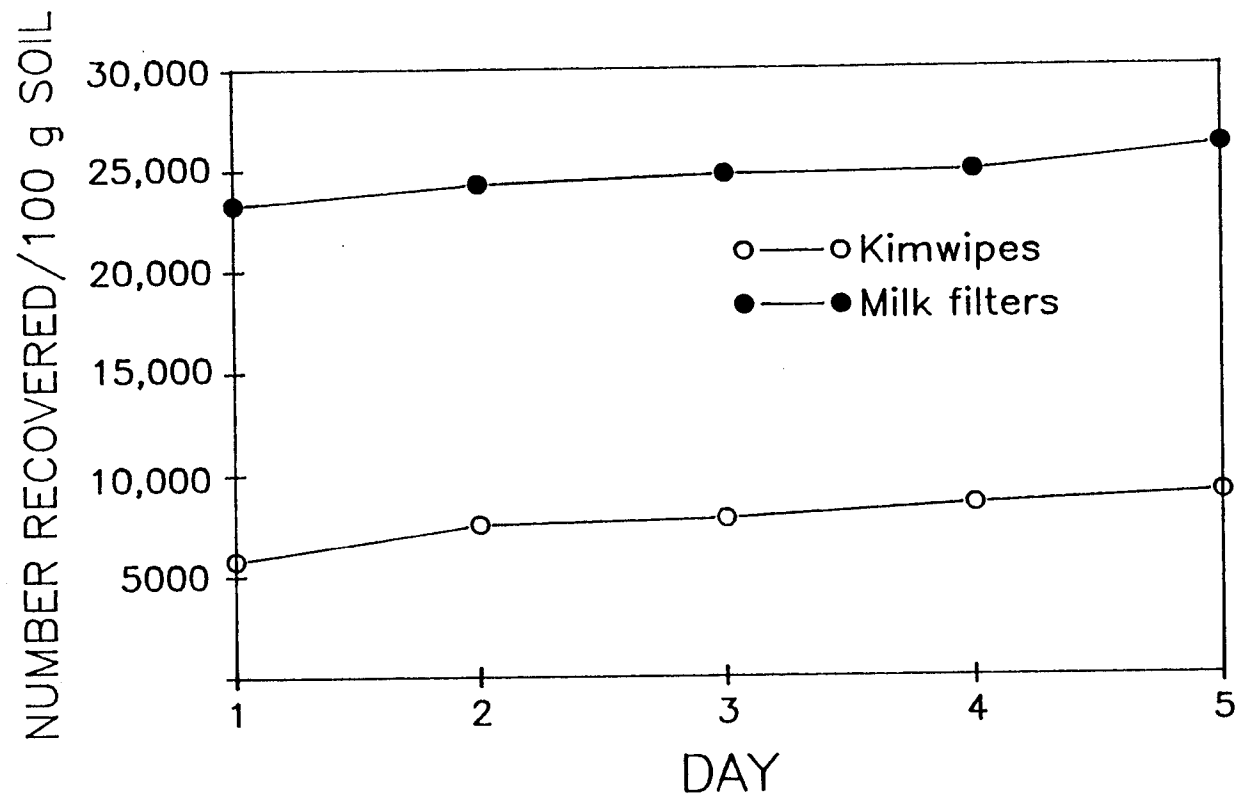


FIGURE 3. CUMULATIVE BAERMANN FUNNEL RECOVERY OF MELOIDOGYNE CHITWOODI

**Experiment 6: Daily recovery of *P. penetrans* from Roots in a Mist Chamber.** Cumulative daily recovery of *P. penetrans* from mist chamber extraction of peppermint roots is illustrated in Figure 4. Nematodes were recovered from mist chamber tubes until day 38 with 12.9% recovered after one day, 73.5% after 7 days (the normal extraction period used in the OSU Plant Clinic) and 91.2% after 11 days.

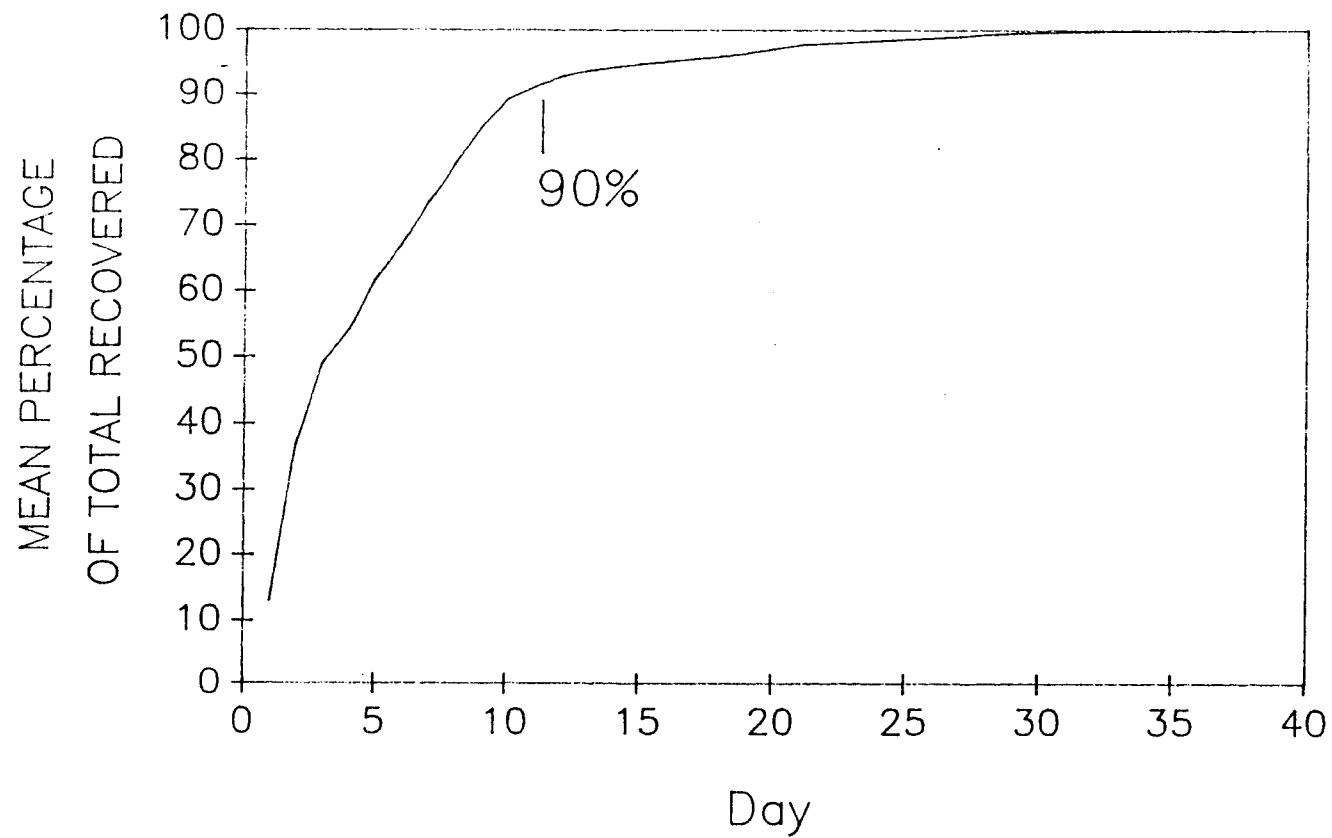


FIGURE 4. CUMULATIVE RECOVERY  
OF PRATYLENCHUS PENETRANS BY MIST CHAMBER EXTRACTION



## DISCUSSION

**WS/SC Efficiency.** In the determination of WS/SC extraction efficiency, percent recovery in the standard sample indicates extraction efficiency. In the five potential sources of loss, two (water and dishpan) involve loss through sieves. Recovery from "water" and from "dishpan" measures percentages of species lost through 38 u and 25 u sieves, respectively. Recovery from the total of "bucket", "pellet I" and "pellet II" measures loss into sediment by either gravitational or centrifugal settling. Recovery of all three species was favorable when compared with similar studies (Williams and Weber, 1968; Viglierchio and Schmitt, 1983c). Significant differences among species among potential sources of loss probably result from differences in nematode characteristics including configuration, ornamentation, and specific gravity.

Extraction efficiency of P. penetrans was 59.1%. Losses through sieves were relatively high (32.1%), while sediment losses accounted for only 8.8% of loss during extraction. This species tends to assume a straight shape, and its juveniles are small. These features may facilitate slippage through even a fine mesh sieve and may

account for the comparatively high 11.5% lost through the 25 u sieve into the dishpan.

Extraction efficiency of Paratylenchus sp. was 80.2%. Sieving losses totalled 9.3%, and sediment losses were 10.4%. This Paratylenchus sp. tends to assume a C shape, which may make it more retainable on a fine sieve, since there is a higher probability that it will fall at an angle across the wires. This may account for the comparatively small percentage lost through both sieving points.

Extraction efficiency of C. xenoplax was 66.1%. Sieving losses accounted for 17.5% and sediment losses 16.4%. Body shape generally remains more straight than curled; thus it may be more likely to pass through a fine sieve than would a curled species. The width to length ratio is higher than that of most nematodes, giving it a lower surface area to volume ratio and therefore a tendency to settle more quickly. While the ornamented cuticle might tend to offset rapid settling by creating more resistance with the water, the percentage lost to sediment is higher than that for either of the other two species, implicating configuration and surface area to volume ratio rather than ornamentation as dominant factors.

Weber and Williams (1968) recovered 35.4% of Hoplolaimus sp., 47.7% of Helicotylenchus sp., and 41.5% of Criconemoides sp. from soil samples in their standard WS/SC recovery process. These percentages represent the relative performance of WS/SC, although direct comparisons of percentage recovery achieved in the present samples are not possible due to species differences. Efficiencies in the present study are somewhat higher than in Williams and Weber's experiments, which may be due in part to the smaller sieve sizes used here.

Sieve pores small enough to efficiently catch small nematodes such as P. penetrans juveniles tend to clog quickly with soil particles. McSorley and Parrado (1981) observed considerable nematode loss through even the smallest sieves (25  $\mu$ ) used, and Hooper (1986) reported pore clogging, as well as loss of nematodes, in sieves of 25  $\mu$  and larger. To avoid clogging inherent in the use of sieves of less than 20  $\mu$ , larger mesh sieves must be used and an extraction efficiency factor determined for each species. This would correct for nematodes lost through sieves without requiring heroic measures such as the use of sieve sizes smaller than 25  $\mu$ , which clog and take longer for water to drain through.

Losses of P. penetrans and Paratylenchus sp. in sediment were near or below 10%, which may be considered

acceptable. Reduction of the relatively high 16.4% loss of C. xenoplax in sediment would be desirable. In comparing sedimentation rates of several nematode species, Viglierchio and Schmitt (1983a) observed the sedimentation rate of C. xenoplax mixed adult and juvenile populations to vary according to age of culture and host plant and that the median sedimentation rate of small nematodes appeared to be less than that of larger ones. Clogging of small pore sieves by small particles may require a settling time that allows some nematodes to settle out along with the smaller particles. Appropriate settling times to use in routine extraction for each species could be investigated, but the resulting separate extractions on the same soil sample for differential recovery of species would not be practical within the time available to most labs. An additional resuspension and wet sieving of sample sediment might be a more practical alternative.

**WS/SC vs BF.** BF recoveries of P. penetrans were nearly the same as those from WS/SC in experiment 2 but significantly higher than WS/SC in experiment 4. The experiment 2 soil was collected on November 12, while the experiment 4 soil was collected on September 1, suggesting that BF is more effective than WS/SC for this species during warm seasons while recoveries from the two methods are similar during cool seasons. However, BF continued to

be more effective than WS/SC throughout the cold storage period. Barker et al. (1969, 1971) found that Baermann funnels recovered the most nematodes during summer and fall when nematodes were active, but centrifugal flotation and sucrose flotation sieving recovered more nematodes in winter and spring.

Significantly more Paratylenchus sp. were recovered by WS/SC than by BF in Experiment 2, but recovery by the two methods did not differ in the long term storage study in Experiment 4. This species is usually efficiently recovered using either method. Significantly more C. xenoplax were recovered by WS/SC in both experiments which compared the two methods for this species. The widely accepted inefficiency of Baermann funnels for extraction of Criconematids (Barker, 1985; Townshend, 1963a; Barker et al., 1969; Viglierchio and Schmitt, 1983c; McSorley and Parrado, 1982) is thus confirmed in these experiments.

Baermann funnel extraction efficiency is indicated by the percentage of each species extracted by BF compared to the BF plus subsequent WS/SC reextraction of BF-extracted soil in experiment 2. P. penetrans and Paratylenchus sp. efficiency, percentages were 58.2 and 57.5, respectively. C. xenoplax BF extraction efficiency was 12.7%, again indicating the difficulty of extracting Criconematids by dynamic extraction methods. However, since WS/SC

extraction of soil without prior BF extraction recovered significantly more nematodes of each species than BF + WS/SC, this procedure for BF efficiency determination would require a correction factor of more than 2, which Viglierchio (1983c) has stated is inadequate.

BF recovers only live nematodes, while WS/SC recovers both live and dead nematodes. Dead but intact nematodes should be recovered in the WS/SC extraction following BF. The difference between the two methods may be due to decomposition of nematodes in the soil sample during BF extraction.

Establishment of a conversion factor for between BF and WS/SC extractions would be desirable for comparing samples extracted by different methods. However, BF to WS/SC ratios for each species differed among the extraction dates in experiment 4. In this case, factors relative to storage could be responsible for producing the different ratios. An accurate conversion figure may vary according to storage time and conditions, the time of year samples are taken, and to the developmental stage of the host plants.

Because recovery of Paratylenchus sp. by WS/SC is usually not significantly different from that by BF, a conversion factor between the two methods is probably not necessary. Recovery of C. xenoplax is so low from BF that

it cannot be considered an appropriate extraction method for this species under any conditions (Viglierchio and Schmitt, 1983c). Thus, a conversion factor from BF to WS/SC would be irrelevant. Barker et al. (1969) also found centrifugal flotation the only effective method for extraction of Criconemoides ornatum, a related species.

**BF Filter Material.** The milk filters routinely used in BF at Oregon State University are soft, and fibers are loosely joined. Kimwipes, however, are denser and have a harder finish. Kimwipes appear to be an unsatisfactory Baermann funnel filter material for the species in this study. On the first extraction date of the M. chitwoodi study (Feb. 24), significantly fewer active juveniles were recovered using Kimwipes than using milk filters, and the nematodes recovered using Kimwipes took longer to move through the filter than those recovered using milk filters. However, after 22 days of cold storage (March 18), recovery from the two different filter types did not differ significantly. On the first extraction date, milk filter and WS/SC extraction methods did not differ significantly in nematode numbers recovered. However, on the second date, recovery from the two different filter types was less than from WS/SC. Some nematodes may have died during storage, but dead nematodes would still be recovered using WS/SC. These results support milk filter

BF as a good indicator of live nematodes and WS/SC as a good indicator of both live and dead nematodes. Kimwipes appear to be inadequate as a filter material for BF.

**Baermann Funnel Daily Recovery:** In daily counts of BF extractions, 90% of Meloidogyne chitwoodi were recovered on day 2 of the extraction when using milk filters. This percentage was not recovered from Kimwipe BF until day 4, further supporting the superiority of milk filters as a BF filter material.

For both P. penetrans and Paratylenchus sp., 90% of the nematodes recovered were obtained by day 3. However, 90% of total C. xenoplax were not obtained until day 5, another indication of the inefficiency of BF to extract C. xenoplax. A three-day Baermann incubation period appears to be adequate for M. chitwoodi, P. penetrans, and Paratylenchus sp., but any length of time may still be inadequate for C. xenoplax.

After one day of BF extraction, 89.1%, 71.2%, 71.7%, and 39.0% of M. chitwoodi, P. penetrans, Paratylenchus sp., and C. xenoplax, respectively, were recovered. Oostenbrink (1960) recovered most Helicotylenchus dihystra and Meloidogyne arenaria from Baermann funnels during the first 24 hours. However, Rodriguiz-Kabana and Pope (1981) found that the extraction rate for Hoplolaimus



galeatus remained high during the first 72 hours and declined thereafter.

**Short Term Storage Time:** There were no significant differences in P. penetrans recovery between the three extraction dates, although there was a downward trend. Paratylenchus sp. recovery increased significantly on the second extraction day and decreased significantly on the third. C. xenoplax recovery increased significantly on the second extraction day and returned to near the original level on the third day. The elapsed days between sampling and extraction thus affect nematode recovery for some species. More study of the increase on extraction day 2 is needed to determine which number is the more representative of nematodes actively affecting plants.

**Long term storage time.** P. penetrans recovery from WS/SC decreased significantly on extraction date 2 (28 days), increased significantly on date 3 (41 days), and again decreased significantly on date 4 (68 days) in Experiment 4. The increase may have been due to hatching and the decrease to subsequent death and decomposition while in storage. Recovery from BF followed this same pattern, but changes were not significant. Barker, et al. (1981) observed an increase in nematodes recovered due to hatching during storage. Emiligy and de Grisse (1970) observed a significant decrease in Heterodera juveniles

and Pratylenchus and Paratylenchus sp. in storage but an increase of Xiphinema bakeri recovery. Pratylenchus eggs occur in soil and also in small fragments of root tissue which are not removed by initial sieving. Egg hatch from these fragments may result in higher nematode recovery from stored soils.

Paratylenchus sp. populations appear persistent in storage for at least 68 days, since no significant differences in recovery between dates were detected by either extraction method. This stability may have been related to the life cycle at the time of sampling, and results could be different at another season. Soil was collected August 9, near the seasonal population peak of Paratylenchus sp. Further study may determine if there is a relationship between seasonality and storage stability.

Recovery of C. xenoplax by WS/SC decreased steadily and significantly over the four long-term storage extraction dates, indicating death and perhaps decomposition of some nematodes. BF recoveries of C. xenoplax also decreased significantly over the four extraction dates. However, the small numbers of this species moving through the filter material are a poor sample of the population, and thus these percentages are considered too small to be a good representation of the population (Viglierchio and Schmitt, 1983c). These

results indicate that both long term and short term storage time can significantly affect nematode recovery and that nematodes should be extracted as soon after collecting as possible.

There were no significant differences between the two extraction dates for Meloidogyne chitwoodi recovered by WS/SC, which recovers both live and dead nematodes. BF/milk filter recovery, which recovers only live nematodes, decreased significantly on the second date. These results suggest that either a significant number of nematodes died but did not decompose during 22 days of storage or that egg hatch equaled decomposition.

**Mist chamber extraction from roots:** Mist chamber extractions of endoparasitic nematodes from roots are routinely left under mist for one week in the O.S.U. Plant Clinic. P. penetrans recovery continued in this study until the 38th day of extraction. Similarly, Wall and Chapman (1967) recovered P. penetrans for 19 days of mist chamber extraction. Thus, nematodes extracted after only one week provide an underestimate of the population. Although Chapman (1957) observed an irregular rate of emergence of endoparasites from roots and found no point in time at which a reliable percentage of the total could be obtained, percent emergence of P. penetrans from mint roots in this experiment was regular and thus predictable,

making feasible a conversion factor based on percent of total recovered on each extraction day. Size and morphology of plant tissue in the mist chamber will affect the speed and thoroughness of the extraction. Wall and Chapman (1967) recovered more nematodes from samples of 3.6 g or less than from larger samples. Root samples in this experiment were usually 2 g or smaller. Smaller samples allow more regular, as well as more thorough, recovery of endoparasites than larger sample.

## SUMMARY

Extraction methods differ in efficiency for different species, and sources of variation are present in each procedure. During cool seasons or after cold storage, Baermann funnels recover more P. penetrans than does WS/SC and is comparable to WS/SC in warmer seasons. BF is much less effective for C. xenoplax. The filter material used in the funnel affects recovery of M. chitwoodi.

Storage time affects extraction efficiency. Nematodes may die or become inactive during storage, or eggs may hatch and increase the population. Even three days of storage can significantly affect the outcome of extraction.

In WS/SC extraction, different species may be lost in different places. Some tend to settle out, whereas others tend to be lost through sieves.

P. penetrans recovery from mist chamber extraction of roots continued for 38 days, and the daily percentage recovered varied little among samples. A one week extraction underestimates the root population of this species.

Further work is needed to understand the mechanisms of extraction procedures used and to determine conversion factors between methods.

Based on this work, WS/SC is the extraction method recommended for C. xenoplax, while either WS/SC or BF is satisfactory for both Paratylenchus sp. and for P. penetrans. There is some indication that BF is more effective than WS/SC in extracting P. penetrans from cooler soils, but further work is needed to confirm this. BF and WS/SC are equally effective for the extraction of M. chitwoodi from soil, but Kimwipes should not be used as filter material in BF. Three days is probably a sufficient BF duration for P. penetrans, Paratylenchus sp., and M. chitwoodi. BF is not recommended for extraction of C. xenoplax.

POPULATION DYNAMICS OF PRATYLENCHUS PENETRANS,  
PARATYLENCHUS SP., AND CRICONEMELLA XENOPLAX  
ON WESTERN OREGON PEPPERMINT

The migratory endoparasitic behavior of root-lesion nematodes presents complex management problems, since individuals in the population are distributed among soil, roots, and rhizomes of peppermint. Routine nematode analyses of soil and roots are done separately, and the weight of roots or rhizomes in a given volume of soil is not determined. Thus, the proportion of the population in soil, roots, or rhizomes and the resulting total population pressure on the plant is unknown. In this study, a standard volume of soil containing peppermint roots and rhizomes was collected and the total number of Pratylenchus penetrans in each component was determined to evaluate the total nematode pressure on the plant as well as the seasonal dynamics of nematode distribution among the three habitats. In addition, soil populations of the migratory ectoparasites Paratylenchus sp. and Criconemella xenoplax were also determined for a standardized volume of soil.

## MATERIALS AND METHODS

**Plot design and sampling:** Two peppermint fields west of Monroe, Benton County, Oregon with high Pratylenchus penetrans, Paratylenchus sp., and Criconemella xenoplax populations and one field near Junction City, Lane County, Oregon with a high density of Paratylenchus sp. were sampled. Five pairs of 6.1 m by 6.1 m plots were established in each of the two Monroe fields. One plot of each pair was treated with 1.1 kg a.i./ha oxamyl, and the other was left nontreated. Each plot was sampled biweekly during spring, summer, and fall, and monthly during the winter from April 1988 to March 1990. The Junction City field was sampled biweekly during the 1988 and 1989 growing seasons. Cores approximately 7.5 cm in diameter and 15 cm deep were collected with a clam shovel. From April through October 1988, one core per plot was taken on each sample date, and for the remainder of the study period, five cores per plot were collected and bulked into a single sample.

**Extraction and counting:** Cores from each plot on each sampling date were sieved through a 5 mm sieve, separating mint roots and rhizomes from soil. Within 36



hr, roots and rhizomes from each plot were suspended on a 5 mm sieve and washed clean of soil and debris in a pressurized water stream from a hand held nozzle. Roots were trimmed from rhizomes and processed as described below. Rhizomes were trimmed of decomposing debris and cut into approximately 6 mm segments. Roots and rhizomes from each sample were weighed separately and placed in an intermittent misting chamber for seven days. Extracted nematodes were removed from the mist chamber, poured into bottles, and either counted immediately or stored at 3.8 C for later counting. The soil was mixed thoroughly, and nematodes were extracted from a 100 g sample using the wet sieving/sucrose centrifugation method described in chapter 1. A 20 g sample from each plot was oven dried (100 C) for at least 8 hr and weighed to determine soil moisture.

Nematodes extracted from soil samples were left to settle in conical-bottomed centrifuge tubes for at least 24 hours. Excess water was decanted and the remaining water and nematodes were poured into a counting dish. Nematodes were counted until 50 - 100 of each of the three species studied were observed. These numbers were multiplied by a conversion factor based on area of the dish observed to obtain the total nematodes of each species in the sample.

### Conversion of results to nematodes per standard core.

In this study, nematode numbers are expressed in relation to a standardized volume of soil. The use of a standard core for evaluating nematode densities over time and between treatments reduces the variability due to changing soil moisture, differences in the size of cores collected, and changes in root and rhizome biomass in that core over time. In this study, a standard core is defined as 500 g dry soil and the peppermint roots and rhizomes in that soil, which was collected to a depth of 15 cm (Figure 5). This was approximately equivalent to the weight of one of the cores collected. Soil nematode counts from wet soil were corrected for soil moisture to obtain nematodes per 100 g dry soil, and numbers of each species converted to numbers of nematodes per standard core consisting of 500 g dry soil and the peppermint roots and rhizomes included in that soil. Nematodes of each species per 100 g dry soil were multiplied by five to obtain nematodes per 500 g dry soil. Fresh root and rhizome weight per gram dry soil collected was multiplied by 500 to obtain the fresh root and rhizome weight that would be present in a standard core of 500 g. P. penetrans per gram fresh root and per gram fresh rhizome was multiplied by these factors to obtain the total P. penetrans in roots and rhizomes from each standard core. Total P. penetrans in soil, root, and

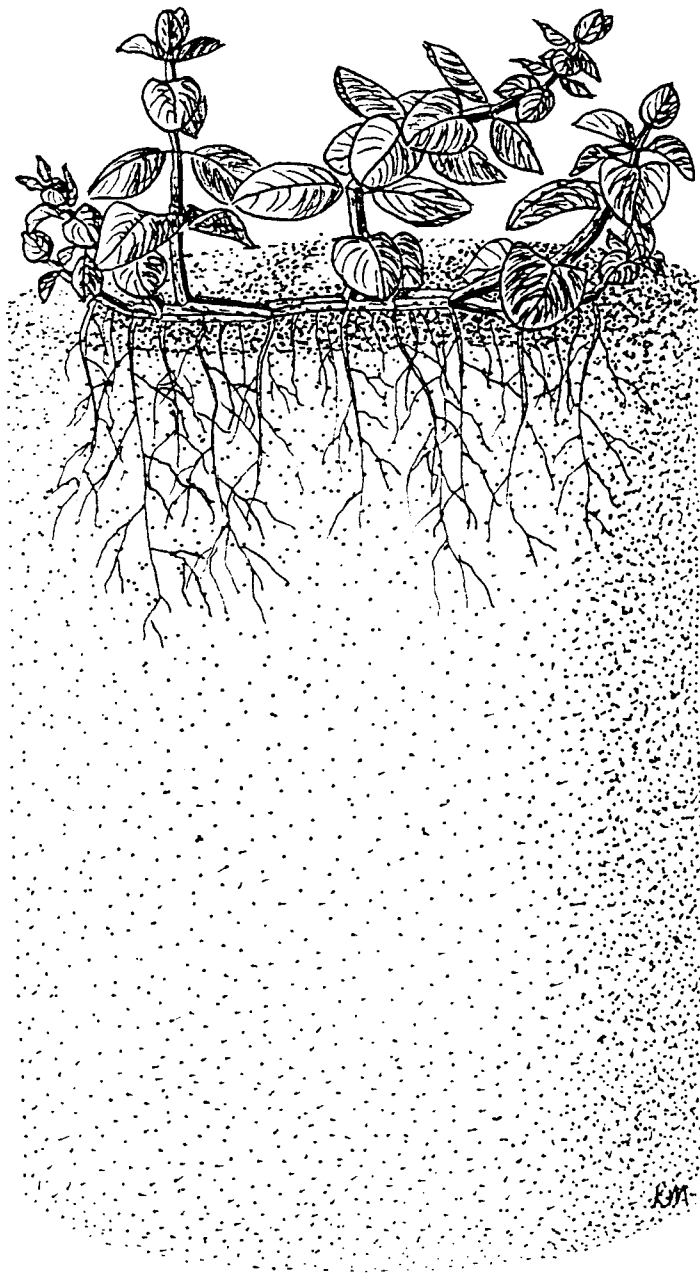


FIGURE 5. THE HYPOTHETICAL STANDARD CORE,  
CONSISTING OF 500 g DRY SOIL PLUS ROOTS AND RHIZOMES  
CONTAINED THEREIN

rhizome components were summed to determine total population in each standard core. Percentages of P. penetrans in soil, roots, or rhizomes for each sampling date were calculated by dividing the number in each component by the total number in the standard core.

**Area under the nematode population curve (AUNPC).**

Area under the two-year curves of P. penetrans in soil, roots, rhizomes, and total standard core; Paratylenchus sp., and C. xenoplax per standard core for each plot were calculated according to the summation over all dates of the expression

$$(X_2 - X_1) * (Y_2 - Y_1) / 2$$

where  $X_2$  and  $X_1$  are consecutive dates and  $Y_2$  and  $Y_1$  are mean nematode numbers on those dates (Shanner and Finney, 1977). Where no significant difference was AUNPC was observed between treated and nontreated plots according to Student's t-test, values from both treatments were averaged and graphed. Population curves from treated and non-treated plots between which significant differences were observed were graphed separately.

## RESULTS

**Soil moisture.** Percent soil moisture varied from 35 to nearly 45 percent during late fall, winter, and early spring, and was lower and more variable during the growing season due to irrigation and intermittent rainfall (Figures 6 and 7). Nematode population changes corresponded poorly to soil moisture fluctuations in all three fields.

**Root weight.** Mean peppermint root weight per standard core peaked in early April of both years at 2.14 to 1.63 g/500 g soil and again in early August at 1.41 to 2.53 g/500 g soil (Figure 8). Mean root weight per standard core of 0.4 to 1.0 g/500 g soil was lowest in late May to early June and in the fall. Decreases in root weight followed flaming in the spring and fall harvest and flaming. Root weight was lower in field 2 than in field 1 (fields near Monroe) during most of the study period, but the difference between these fields was more pronounced during the 1988 growing season and the following winter.

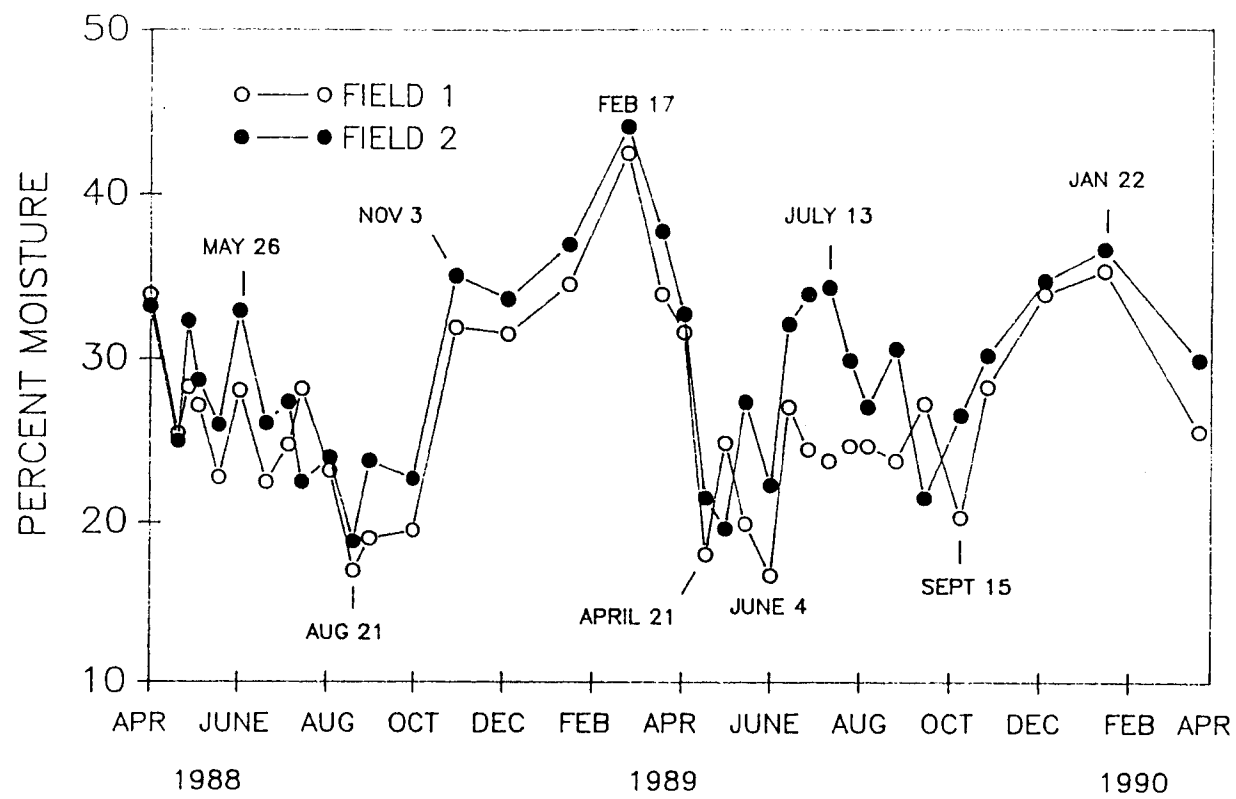


FIGURE 6. SOIL MOISTURE, FIELDS 1 AND 2

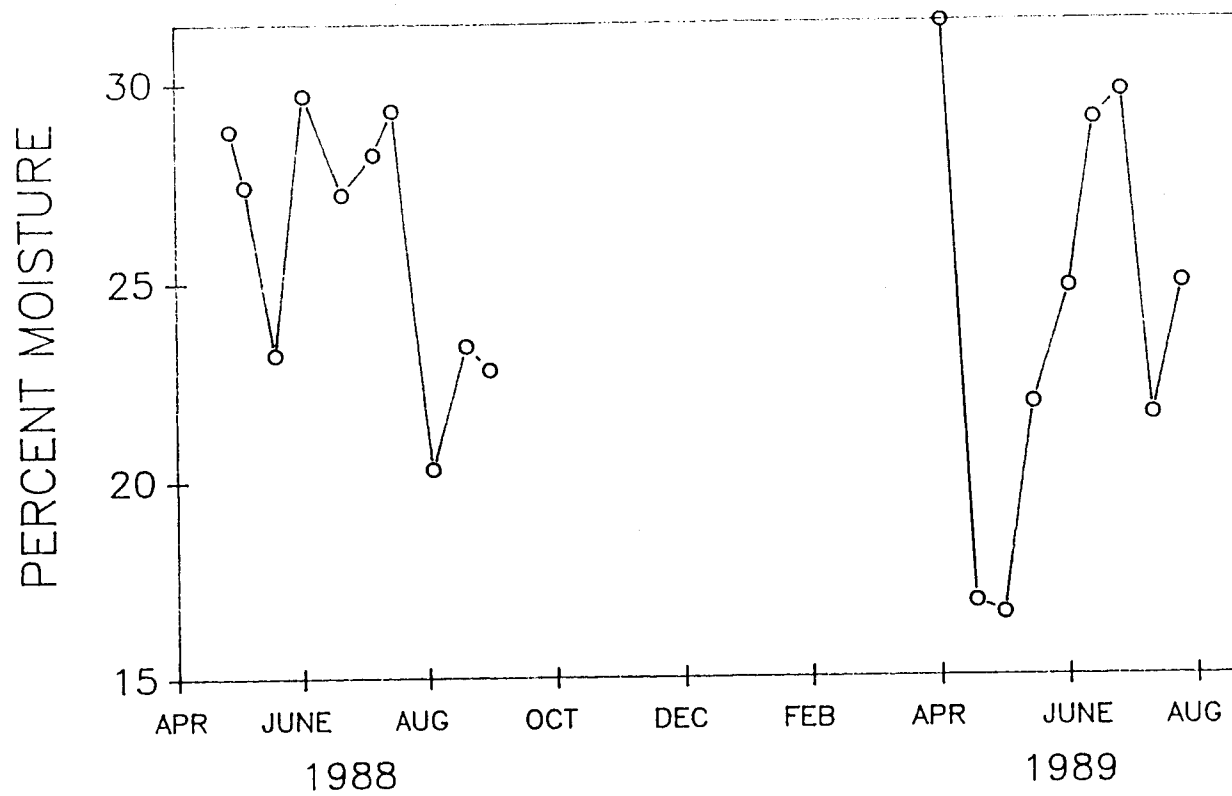


FIGURE 7. SOIL MOISTURE, FIELD 3

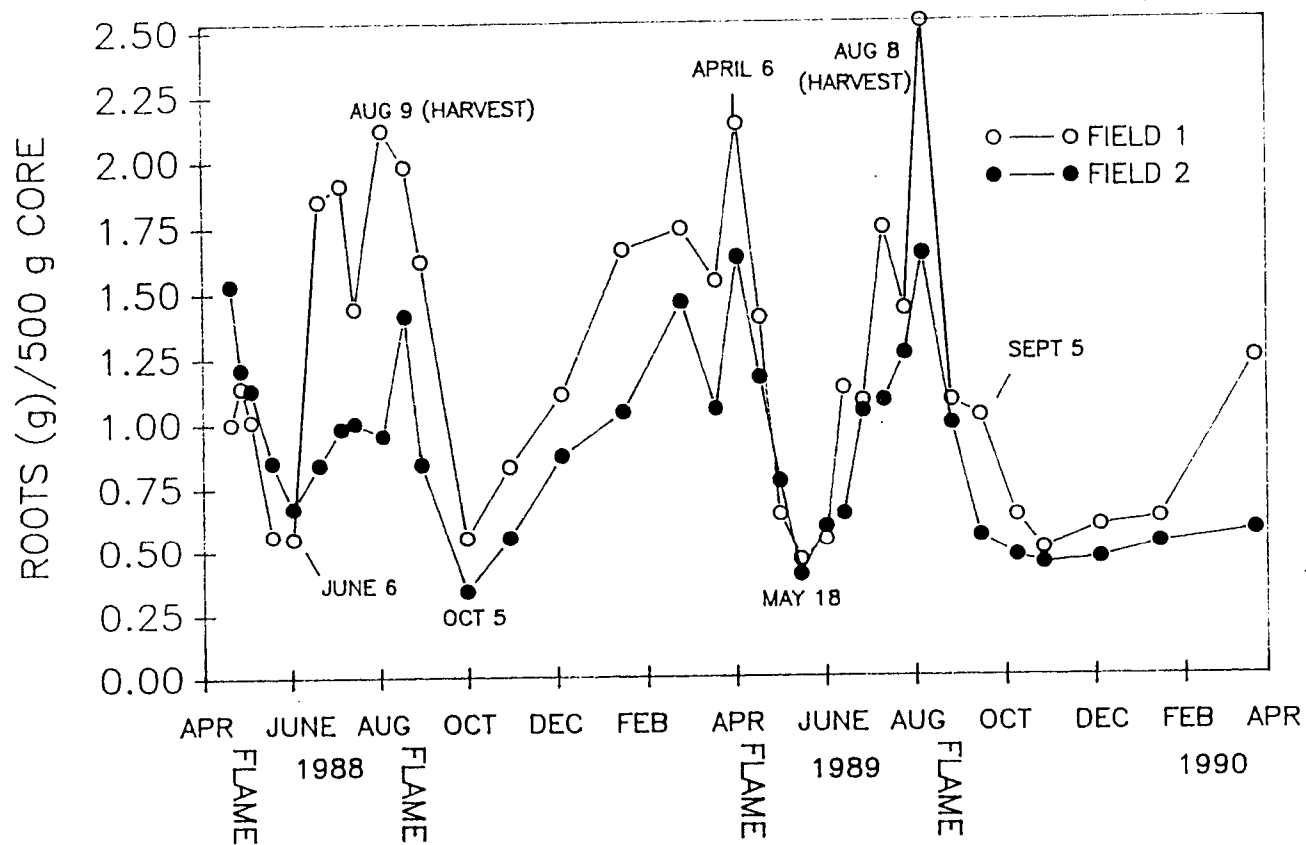


FIGURE 8. STANDARD CORE ROOT WEIGHT, FIELDS 1 AND 2



**Rhizome weight.** Mean rhizome weight per standard core followed the same pattern as roots, although maxima of 4.34 to 8.34 g/500 g soil were usually less pronounced and occasionally occurred earlier than root weight peaks (Figure 9).

**AUNPC Determination.** Significant treatment differences between AUNPC ( $p = 0.05$ ) were observed for P. penetrans in standard core soil and rhizomes. P. penetrans in standard core soil and rhizomes are thus separated by treatments in graphs. In addition, significant field differences between AUNPC ( $p = 0.05$ ) were observed between fields for P. penetrans standard corer rhizomes and for C. xenoplax per standard core.

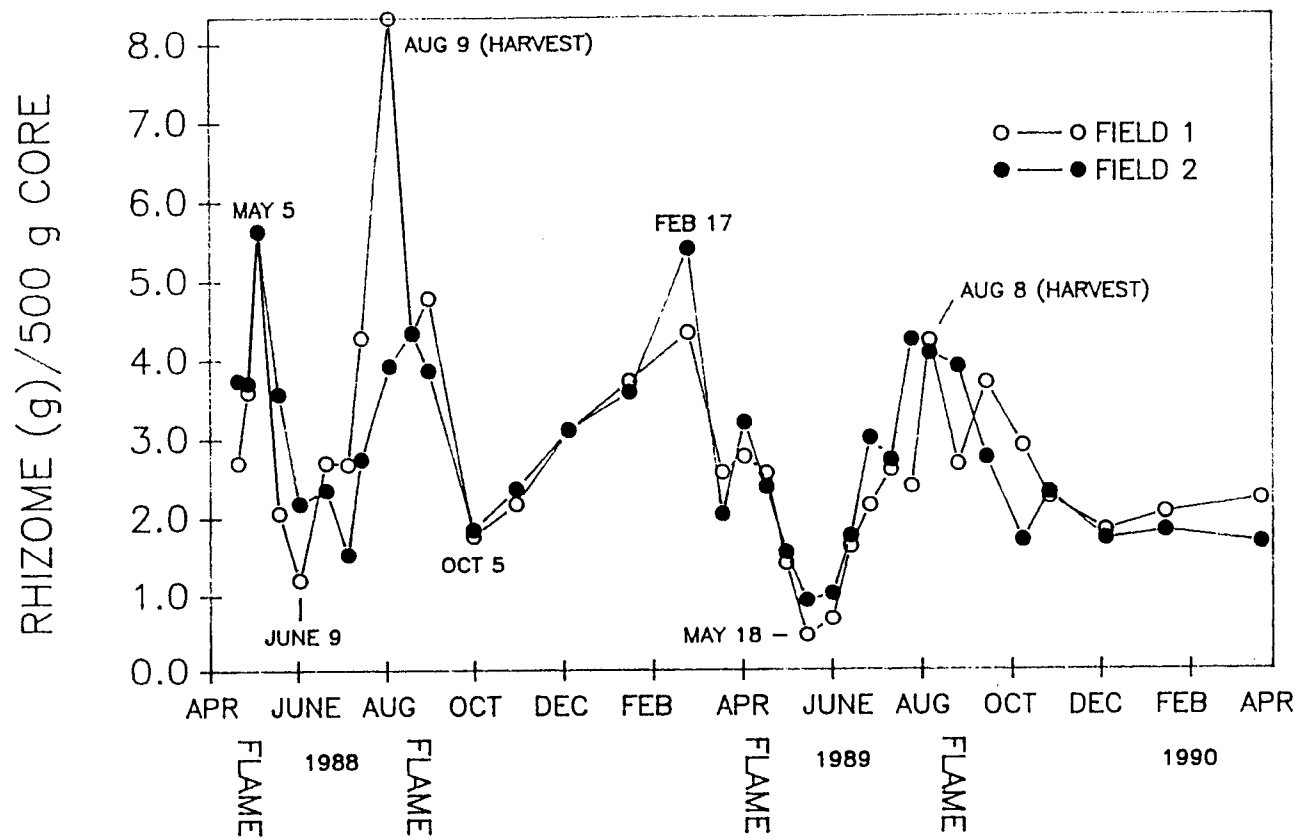


FIGURE 9. STANDARD CORE RHIZOME WEIGHT, FIELDS 1 AND 2

P. penetrans per standard core. Population dynamics of P. penetrans per standard core (soil, roots, and rhizomes) fluctuated similarly to root weight with population peaks following root weight peaks by two to four weeks (Figure 10). Populations in both fields during both years were relatively high in early May (690 to 3441/standard core), declined to a summer plateau of less than 500/standard core in 1988 and 1000 in 1989, increased again in late August to early September to between 1100 and 2500, and declined again to a winter plateau of below 500/500 standard core in 1988 and usually below 1000/standard core in 1989. Winter densities were lower during the harsh winter of 1988 than during the mild winter of 1989. A population minimum in late March, 1989 followed about eight weeks after the early February hard freeze. Maxima in late August and early September were higher following the 1989 growing season than following the 1988 growing season.

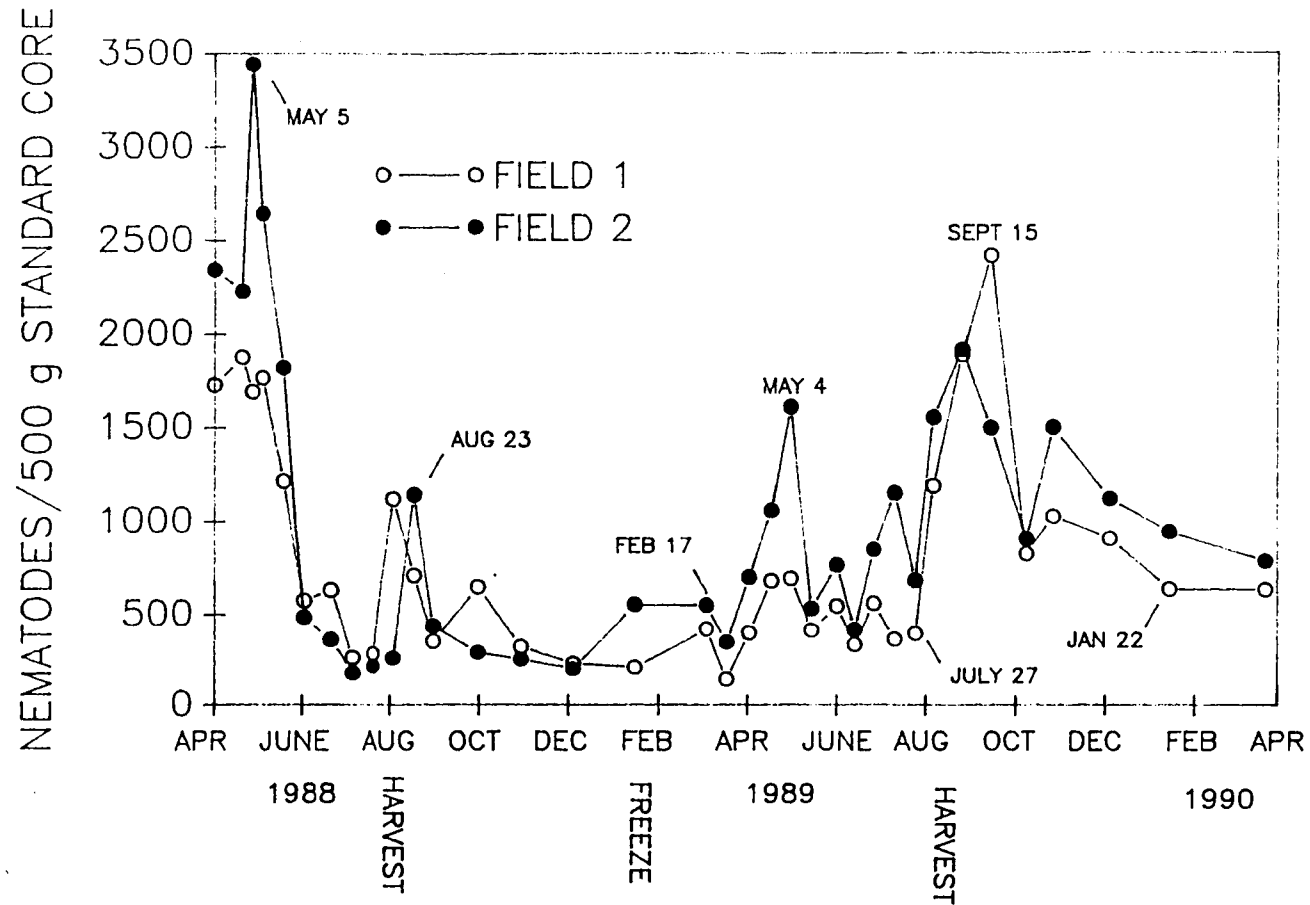


FIGURE 10. PRATYLENCHUS PENETRANS IN STANDARD CORE, INCLUDING SOIL, ROOTS, AND RHIZOMES

**P. penetrans in standard core soil.** Population dynamics of mean P. penetrans in soil alone in nontreated plots followed similar patterns in fields 1 and 2 with peaks of approximately 600 to 800/500 g in late May, 1988; 500 to 900/500 g in late September or early October, 1988; peaks of 400-500/500 g in both fields in late May, 1989; and peaks of 1800/500 g in field 1 and 1240 in field 2 in late August, 1989 (Figures 11 and 12).

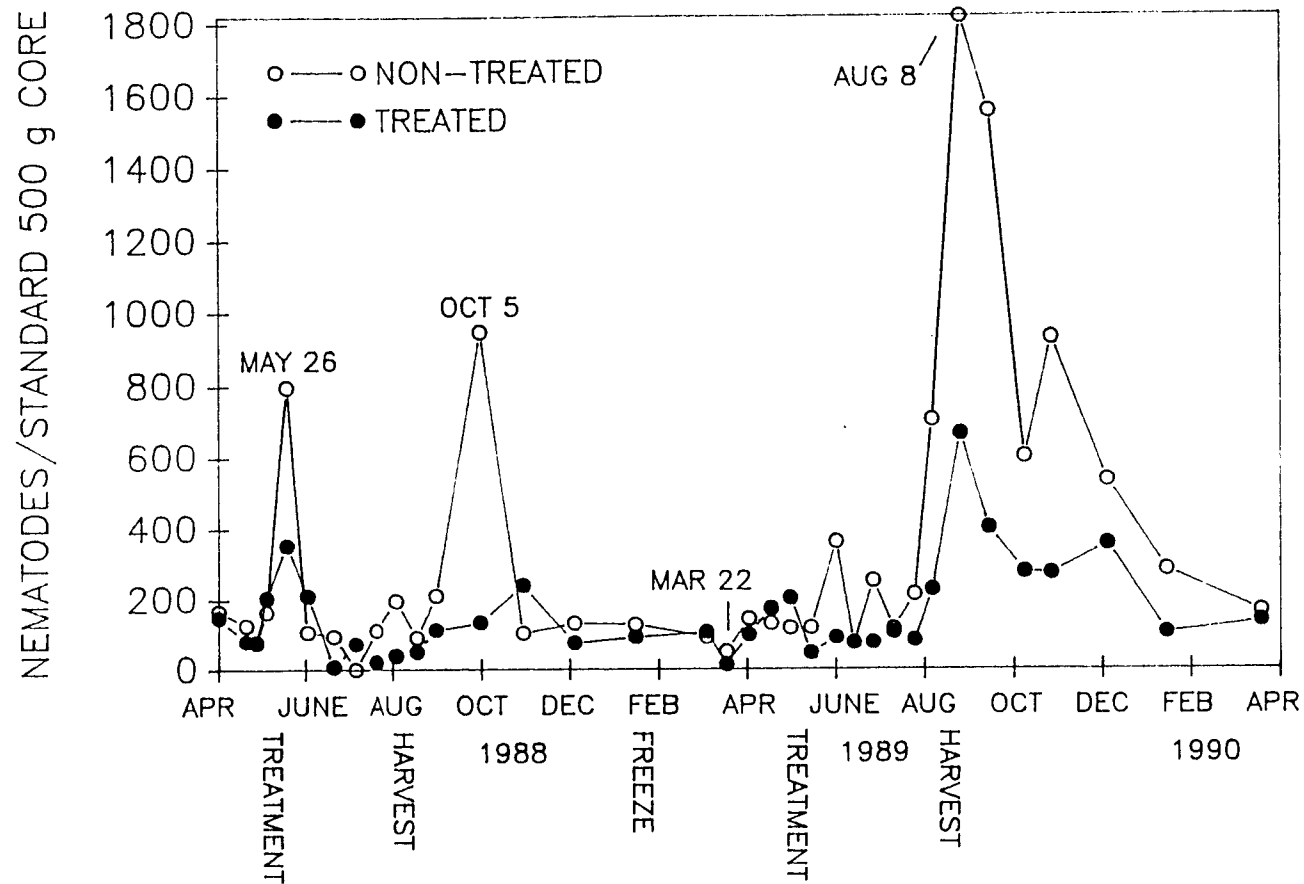


FIGURE 11. PRATYLENCHUS PENETRANS  
IN STANDARD CORE SOIL, FIELD 1

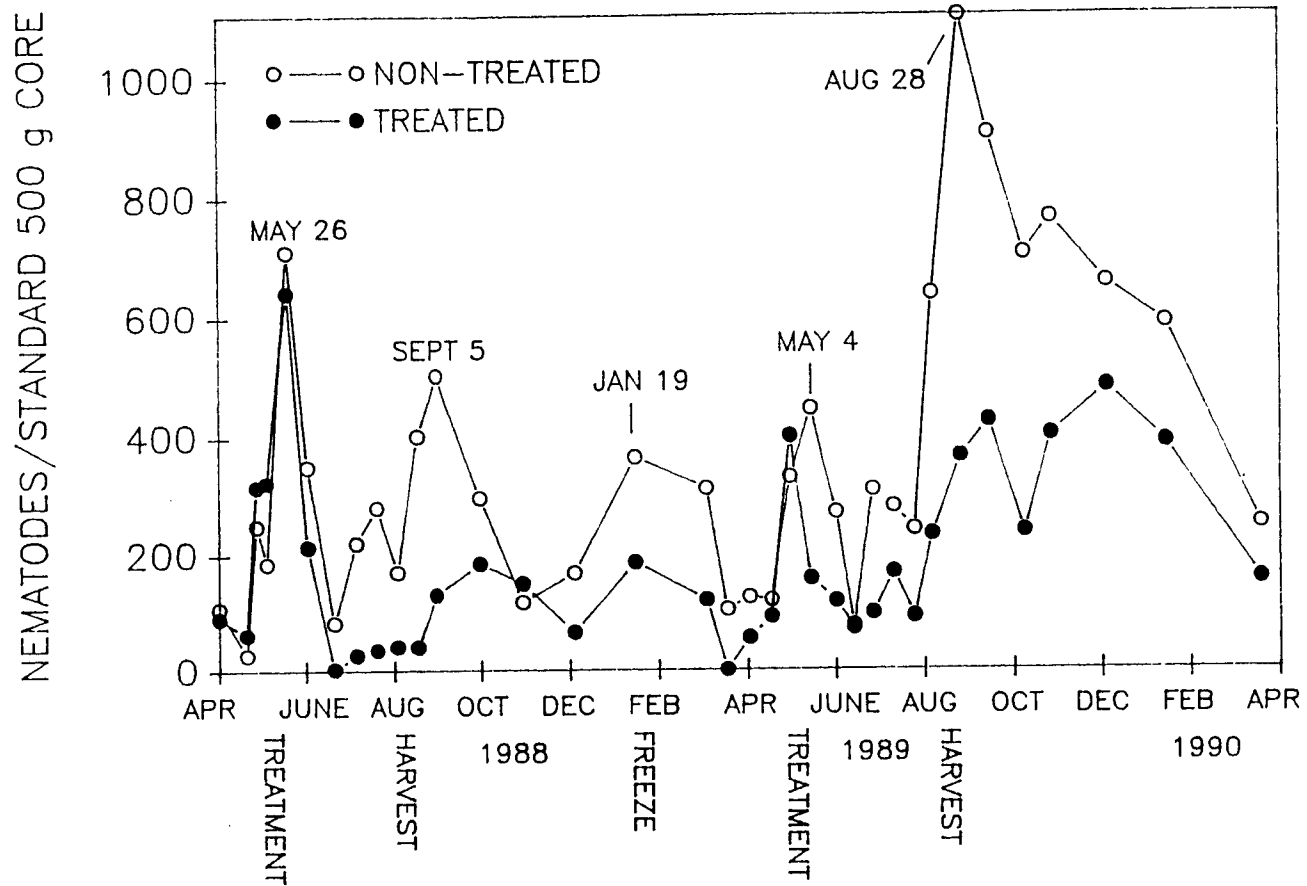


FIGURE 12. PRATYLENCHUS PENETRANS  
IN STANDARD CORE SOIL, FIELD 2

P. penetrans in standard core roots. Root population fluctuations were also similar between fields 1 and 2, although the population in field 2 was higher and more variable than that in field 1 during the 1989 growing season (Figure 13). Peaks of 900 to 2240 nematodes/standard core from roots in a standard core occurred in early May and in late August or September, while summer and winter populations were below 100 per standard core in 1988 and below 600/standard core in 1989.



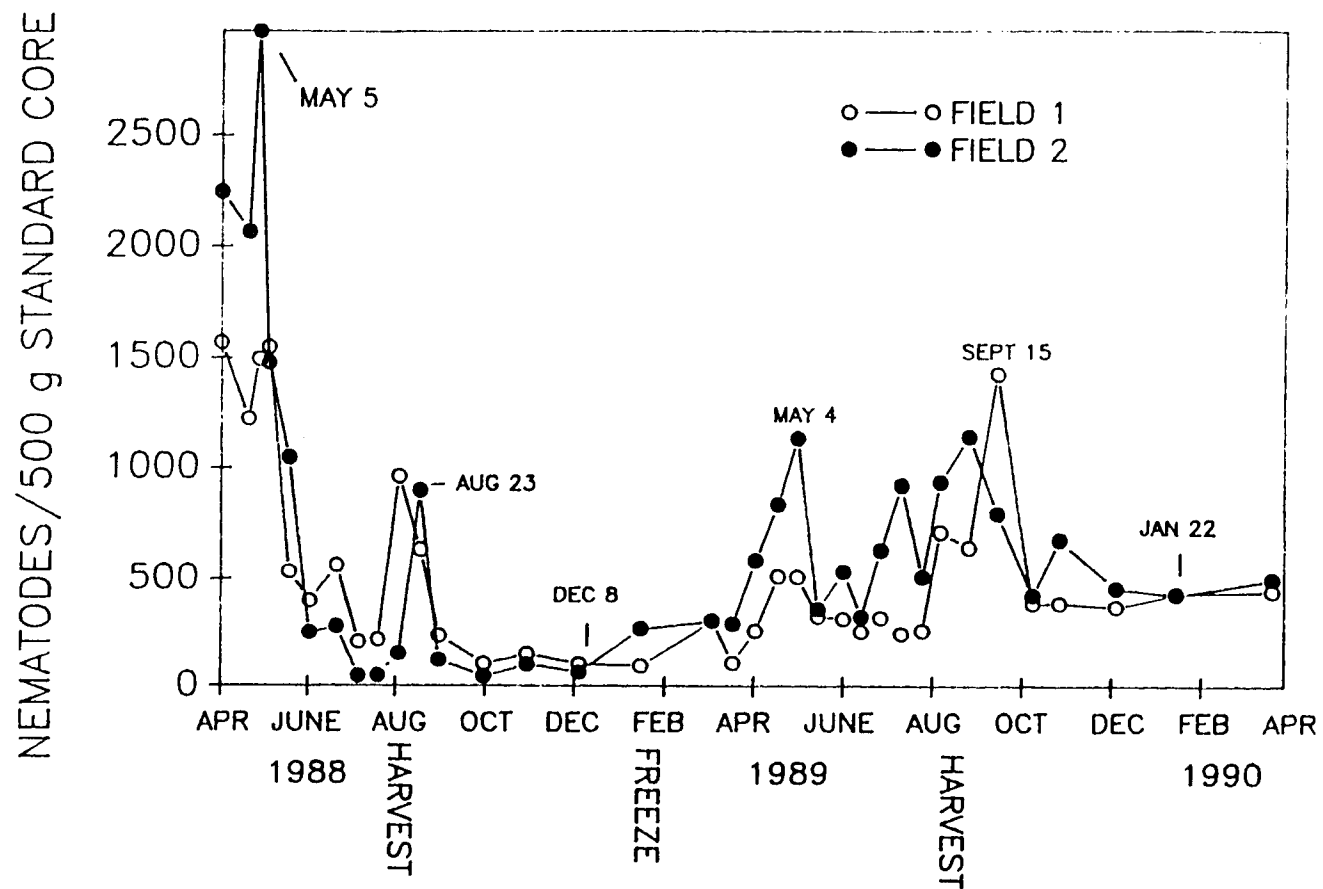


FIGURE 13. PRATYLENCHUS PENETRANS  
IN STANDARD CORE ROOTS, FIELDS 1 AND 2

P. penetrans in standard core rhizomes. Population densities in standard core rhizomes fluctuated markedly throughout the two year study period (Figures 14 and 15). The widest fluctuations usually occurred during the summers, while populations were comparatively stable in the winter during both years. Population changes were similar between treatments within field 1 and field 2, with populations in treated plots often lower than those in non-treated plots. In both fields, rhizome P. penetrans reached minima in August and October and maxima during December during both years.

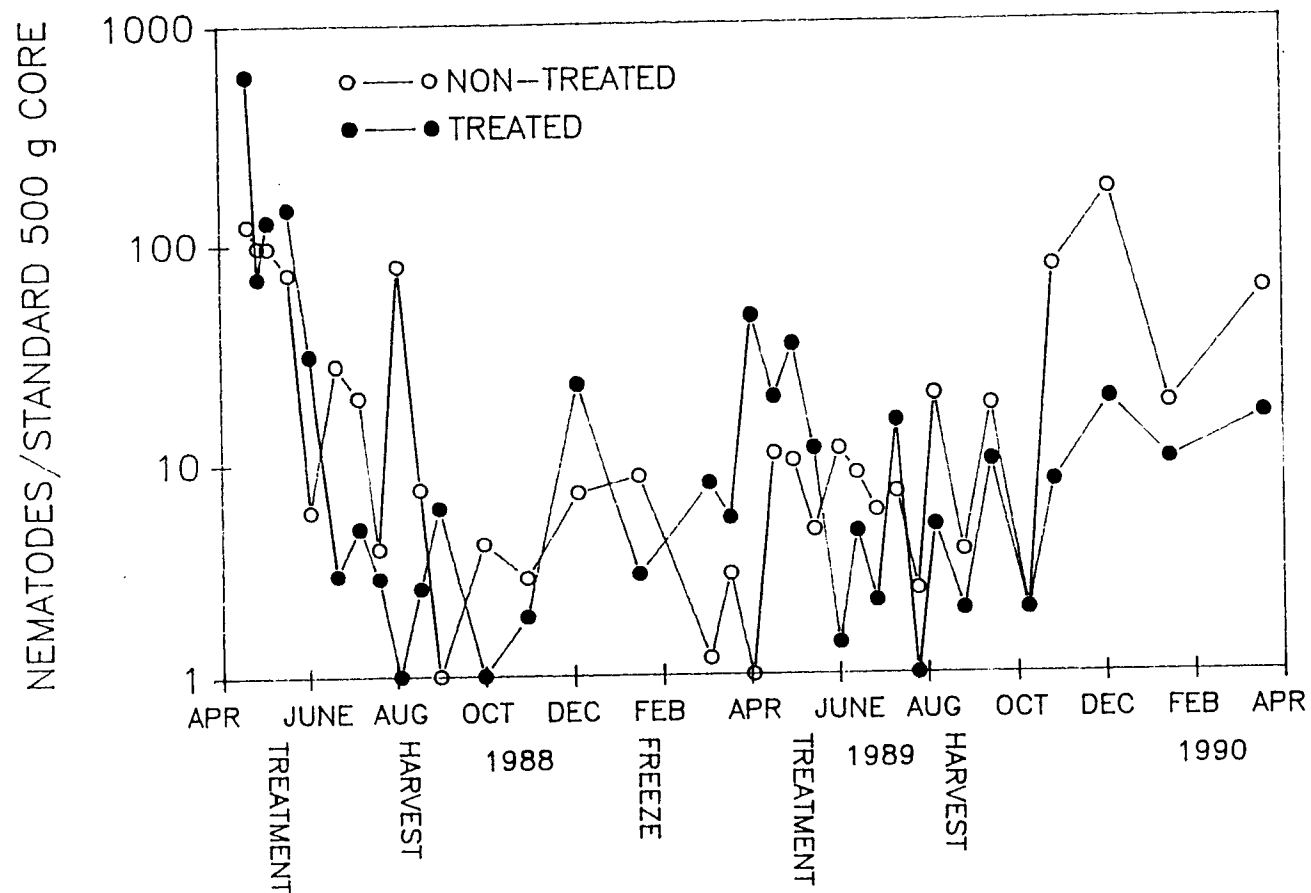


FIGURE 14. PRATYLENCHUS PENETRANS  
IN STANDARD CORE RHIZOMES, FIELD 1

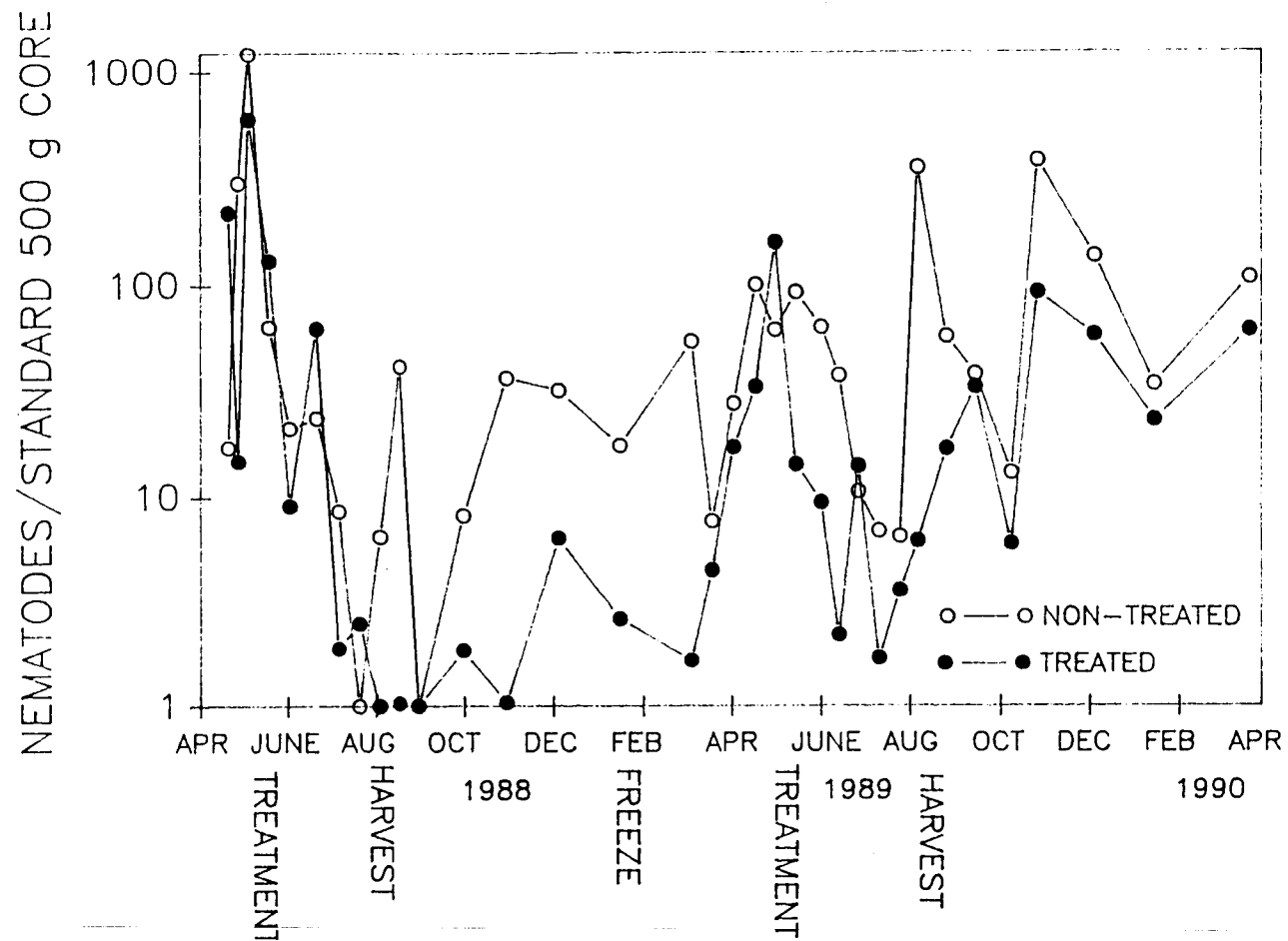


FIGURE 15. PRATYLENCHUS PENETRANS  
IN STANDARD CORE RHIZOMES, FIELD 2

Distribution of P. penetrans populations between soil, roots, and rhizomes. Except in the fall of both years, standard core root populations were larger than soil populations, and the fluctuations in both populations followed the same pattern (Figures 16 and 17). Population peaks occurred at slightly different times in each field. However, soil populations were somewhat higher and root populations were markedly higher during the mild winter of 1989-1990 than during the severe winter of 1988-1989.

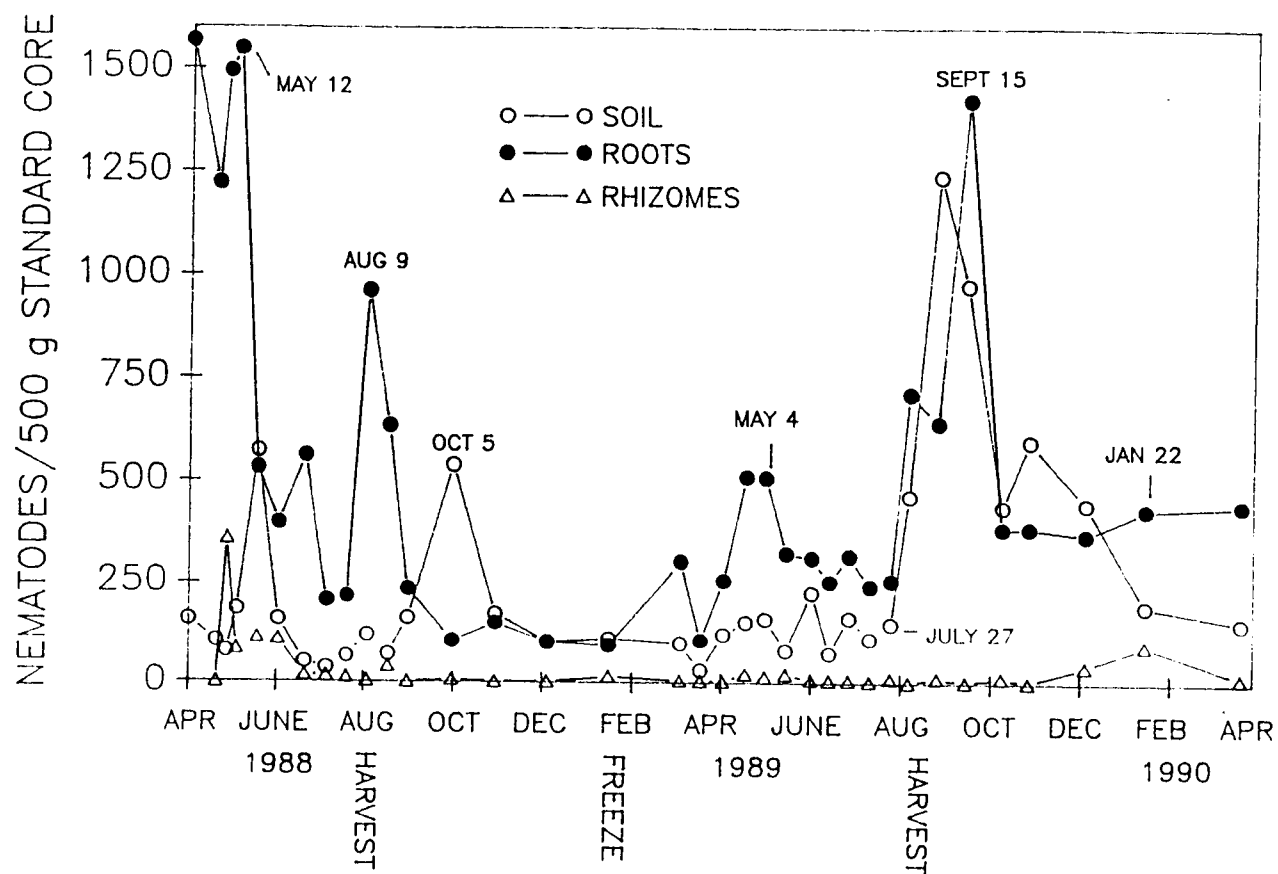


FIGURE 16. PRATYLENCHUS PENETRANS IN STANDARD CORE:  
SOIL, ROOTS, AND RHIZOMES, FIELD 1

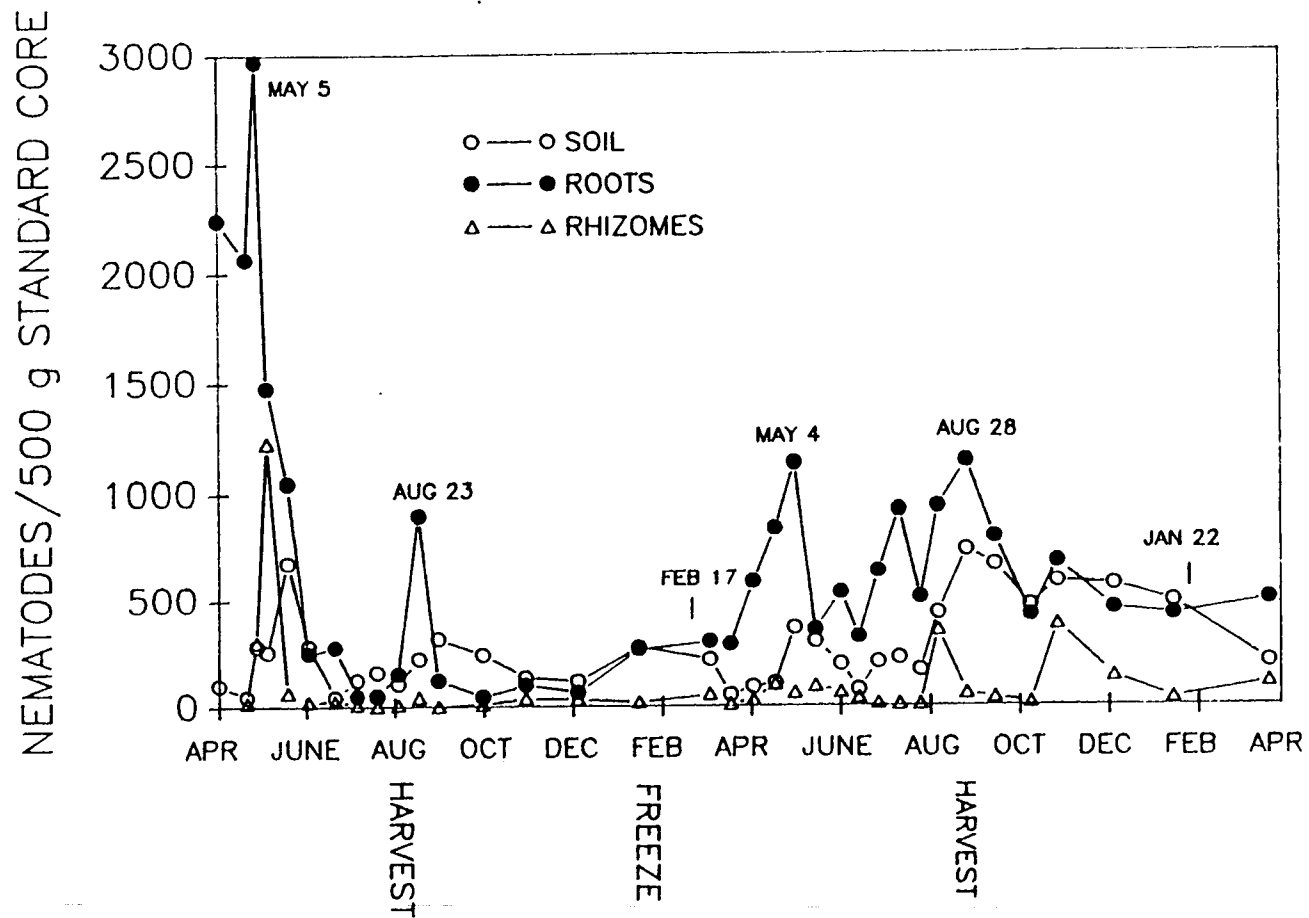


FIGURE 17. PRATYLENCHUS PENETRANS IN STANDARD CORE:  
SOIL, ROOTS, AND RHIZOMES, FIELD 2

From 70 to 90% of the total P. penetrans standard core population was in the roots in early May 1988 (Figures 18 and 19). This percentage decreased to 40 to 50 by the end of June and to 20 to 40% at harvest. From fall to early spring, root populations increased again to 80%. While the percentages of P. penetrans in soil and roots fluctuated during the both growing seasons, the root population of P. penetrans was generally higher. The root proportion of the population increased through the winter, again approaching 70 to 90% in the spring of 1990. More P. penetrans were recovered from roots than from soil on most dates except in late September and early October. High population percentages in roots coincided with high root weights.



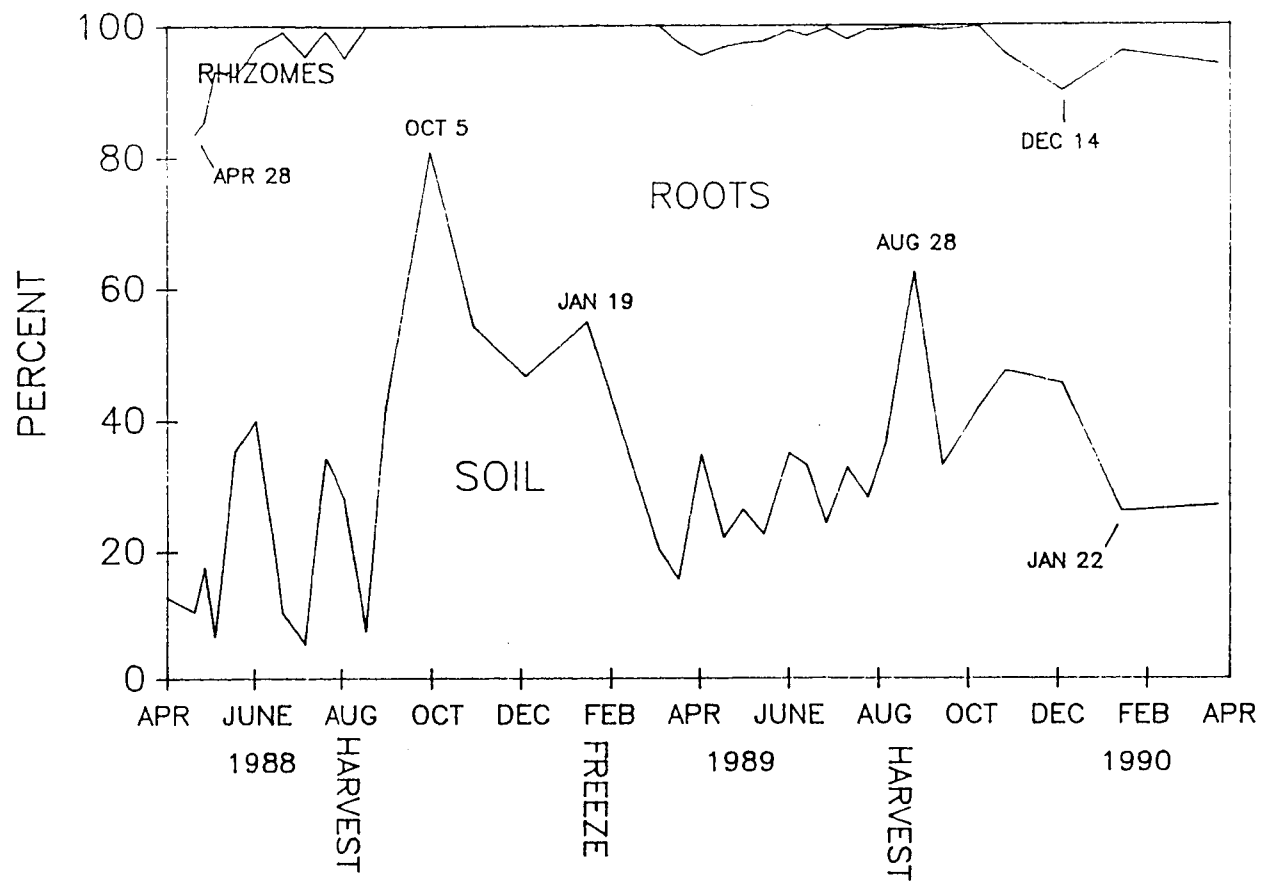


FIGURE 18. DISTRIBUTION OF PRATYLENCHUS PENETRANS AMONG COMPONENTS WITHIN A STANDARD CORE, FIELD 1

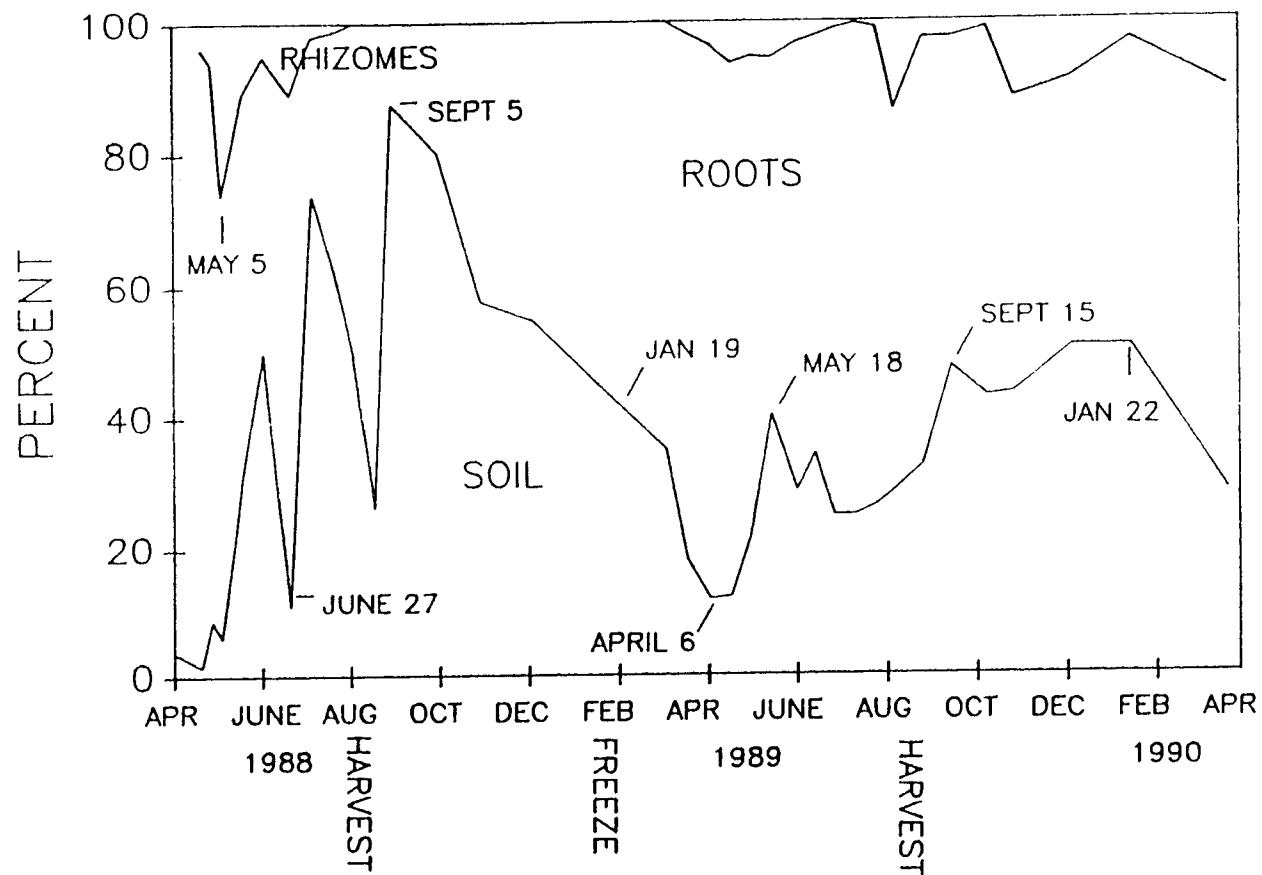


FIGURE 19. DISTRIBUTION OF PRATYLENCHUS PENETRANS AMONG COMPONENTS WITHIN A STANDARD CORE, FIELD 2

Up to 20% of the P. penetrans population occurred in the rhizomes on some sampling dates, but the rhizome percentage was usually not greater than 10%. P. penetrans was present in rhizomes in both fields during the 1988 growing season but was not detected during the winter of 1988-1989. Nematodes were again recovered from rhizomes throughout the 1989 growing season. After a fall decrease in the population, they were again recovered from rhizomes during the winter of 1989-1990.

Paratylenchus sp. per standard core. Paratylenchus sp. populations in fields 1 and 2 were low in winter, increased steadily through spring and summer, and peaked during late August (Figure 20). The August peaks coincided with root weight maxima, but although root weight also peaked in early April, there were no April Paratylenchus sp. peaks. Populations were lower during the severe winter of 1988-1989 than during the following milder winter. A slight spring increase and a trend towards fall increase were detected in the Paratylenchus sp. population in field 3 sampled only during the 1988 and 1989 growing seasons (Figure 21). Peppermint plants in field 3 were fewer and smaller in 1989 due to severe Verticillium wilt, discing, and flaming. Since Paratylenchus sp. populations were markedly lower during 1989 than during 1988, the state of the peppermint plants and the size of the nematode population appear to be related.

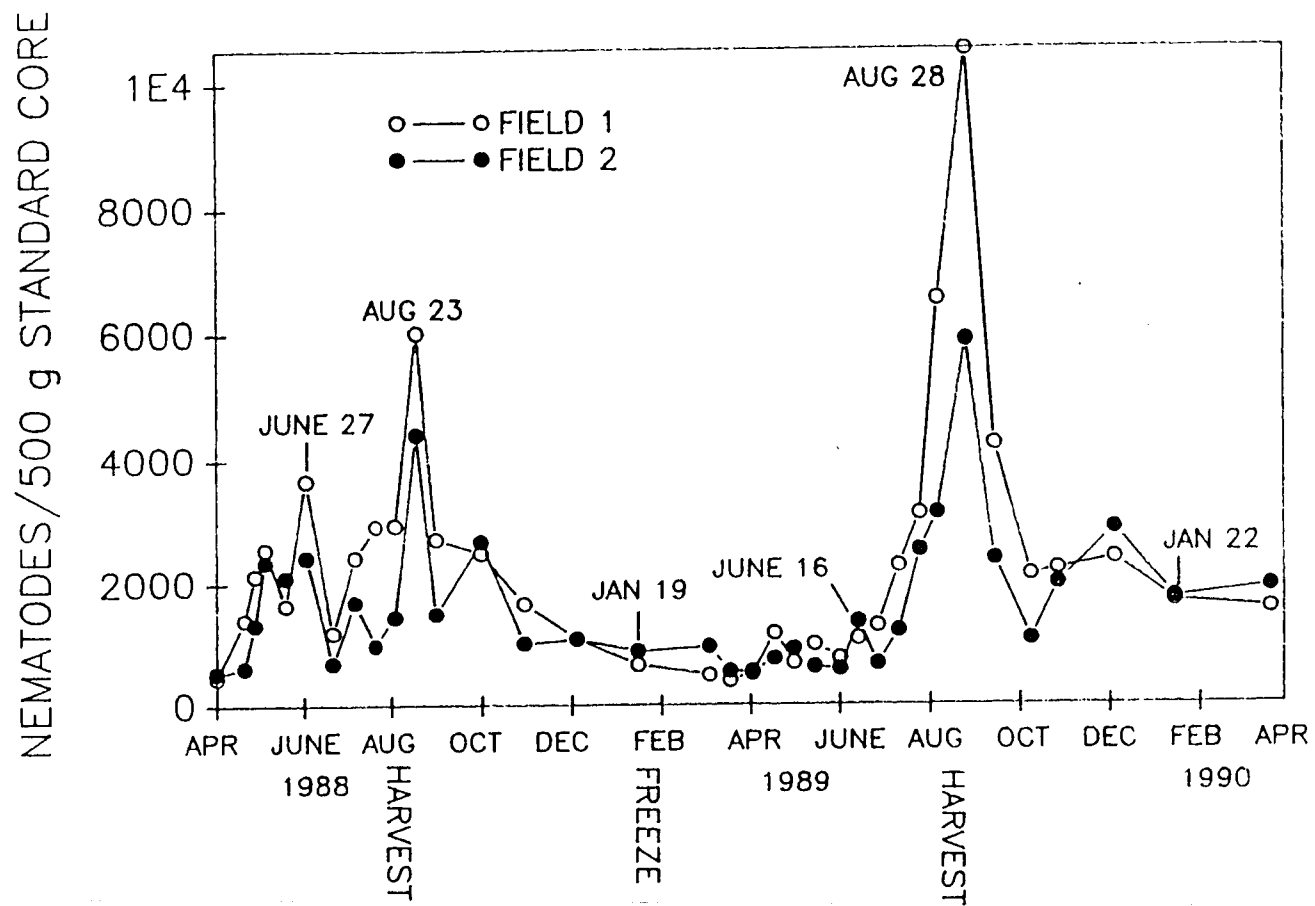


FIGURE 20. PARATYLENCHUS SP. IN A STANDARD CORE,  
FIELDS 1 AND 2

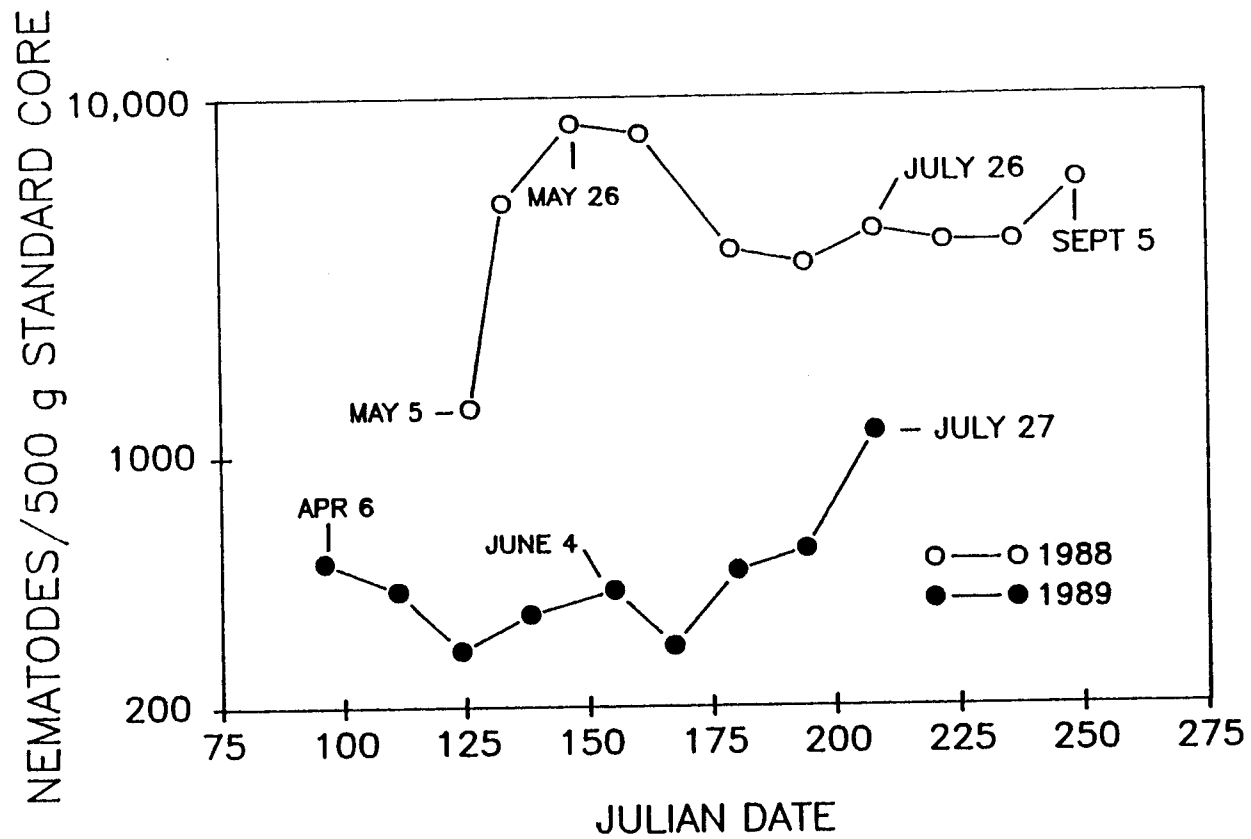


FIGURE 21. *PARATYLENCHUS* SP.  
IN A STANDARD CORE, FIELD 3

C. xenoplax per standard core. High but variable numbers of C. xenoplax were recovered from field 1 throughout the study period. However, in field 2, C. xenoplax was low during 1988 and increased during 1989 (Figure 22). Population highs and lows in fields 1 and 2 corresponded in some cases. The wide density fluctuations did not appear to coincide with root growth or soil moisture.

P. penetrans populations were lower than those of Paratylenchus sp. in both field 1 and field 2 throughout the study period except for the early May population peak (Figures 23 and 24). C. xenoplax levels fluctuated between those of the other two species. All three species peaked in late August, following the early August root weight maximum.

NEMATODES/500 g STANDARD CORE

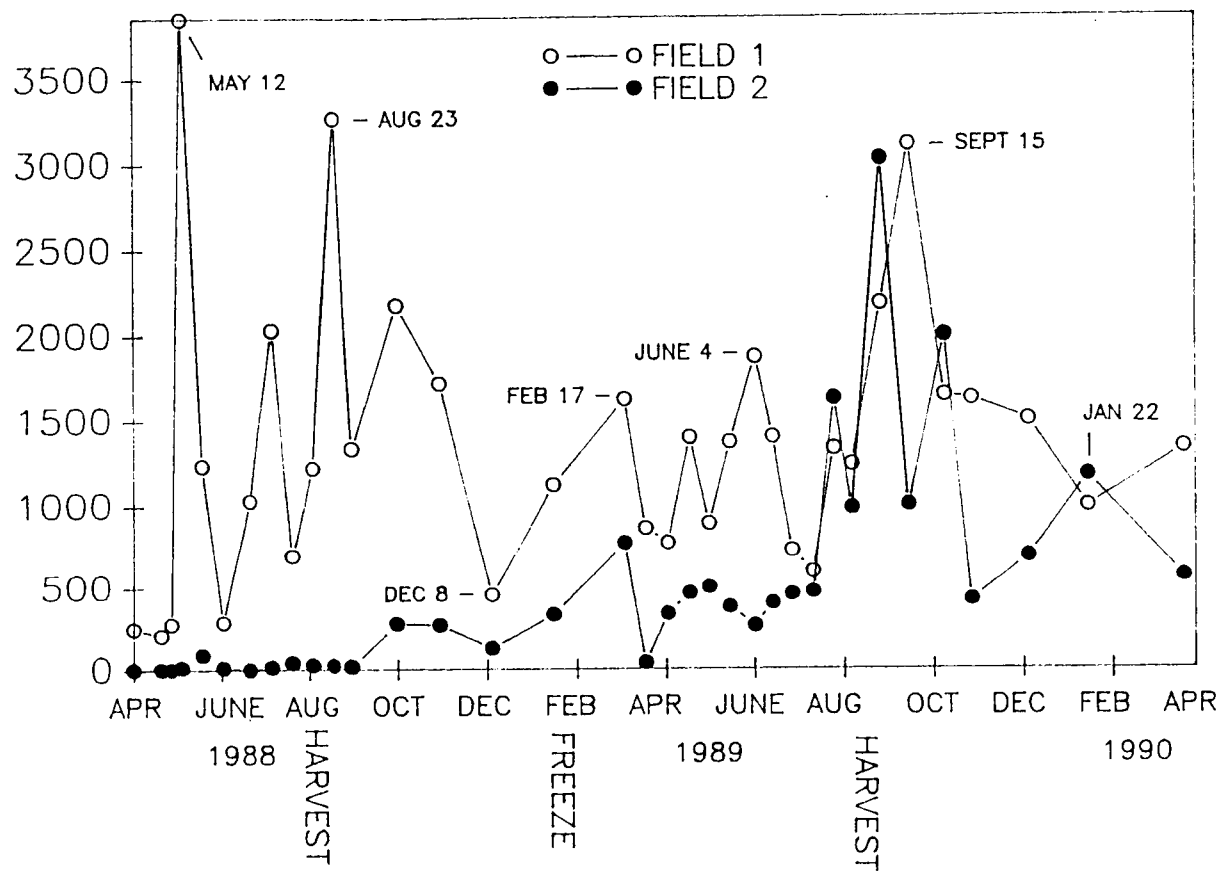


FIGURE 22. CRICONEMELLA XENOPLAX IN A STANDARD CORE, FIELDS 1 AND 2



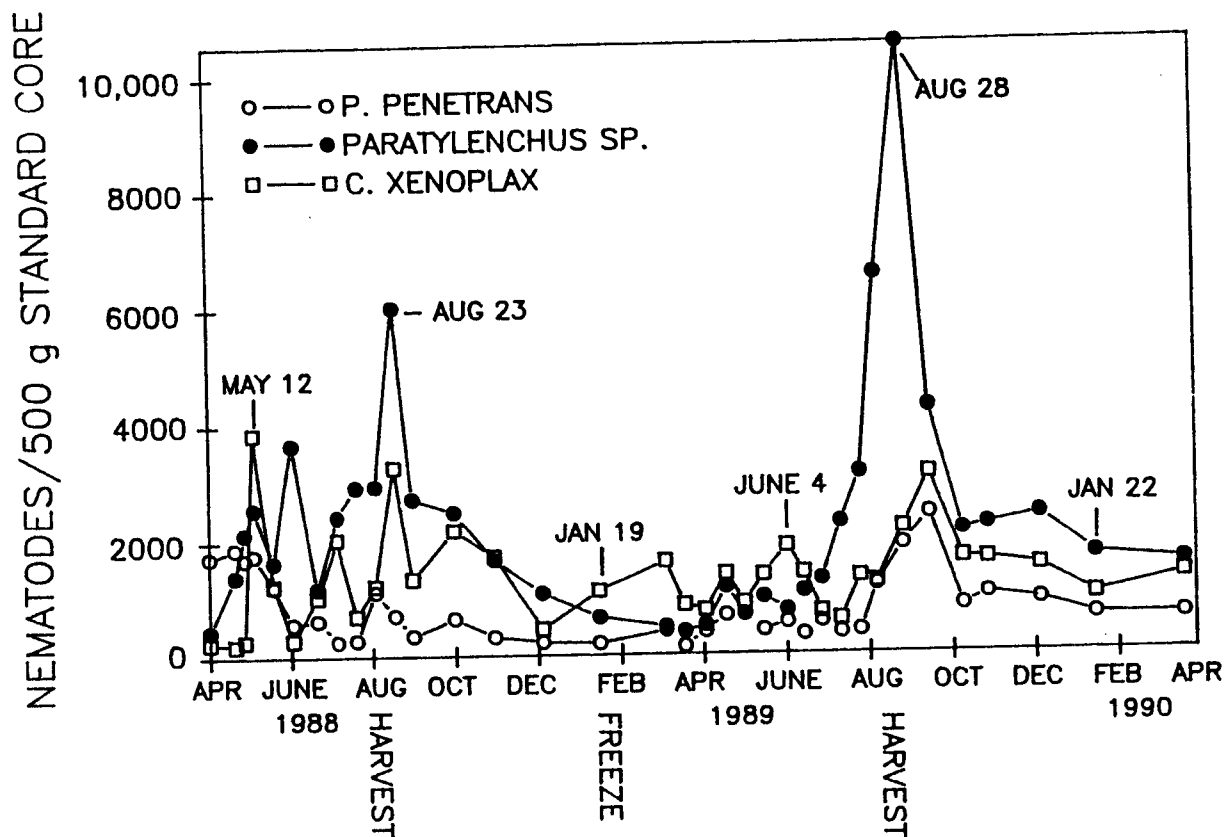


FIGURE 23. PRATYLENCHUS PENETRANS, PARATYLENCHUS SP., AND CRICONEMELLA XENOPLAX IN A STANDARD CORE, FIELD 1

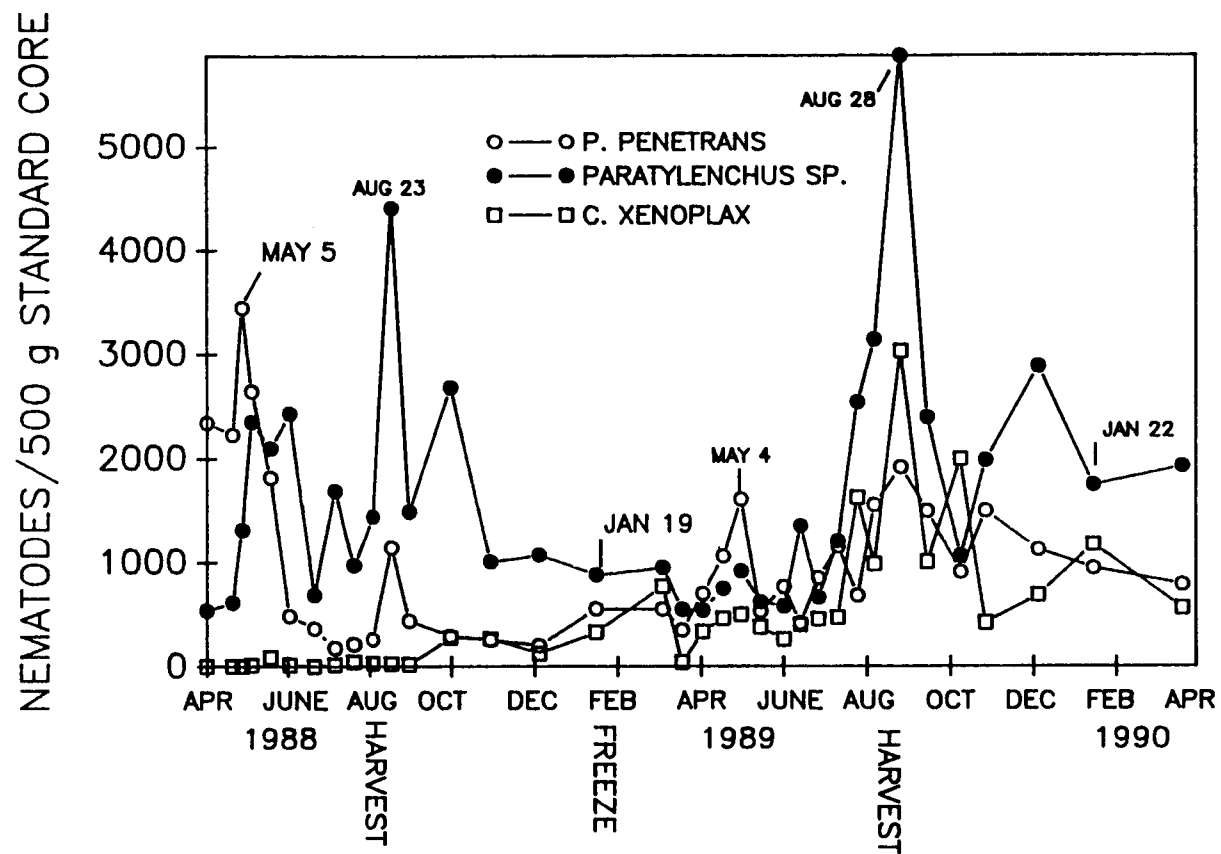


FIGURE 24. PRATYLENCHUS PENETRANS, PARATYLENCHUS SP., AND CRICONEMELLA XENOPLAX IN A STANDARD CORE, FIELD 2

## DISCUSSION

**Standard core evaluation.** Endoparasitic nematode analyses can be determined in terms of soil numbers, root numbers, or both. Results are usually expressed as the number of nematodes in a weight or volume of soil and the number per weight of roots. Using this traditional procedure, the total population pressure on the plant is still unknown, since the weight of roots per volume of soil is not determined. The standard core method addresses this problem by collecting a core of soil which still contains the roots (and the rhizomes, in the case of peppermint). Total numbers of nematodes in soil, in roots, and in rhizomes of that core are then determined. Since core size varies, data are standardized based on a core weighing 500 g (e.g., 7.5 cm in diameter and 15 cm deep). Expressing data in terms of nematodes in 500 g of soil, in the roots and in the rhizomes contained in that soil at the time of collection as well as in the total core provides a measure of the total endoparasitic nematode pressure on the plant. Expressing numbers of migratory ectoparasitic nematodes in terms of the standard core, i.e., in 500 g soil, provides a measure of ectoparasitic nematode pressure on the plant that is

comparable to that for endoparasites. The standard core concept also permits evaluation of root and rhizome weight on a comparable basis.

The relationship between nematode numbers in soil, roots, and rhizomes is dynamic and appears to vary, sometimes dramatically and rapidly, during the year. Thus, results from a soil or root sample only will vary according to the time of year the field is sampled, and management decisions may be adversely affected by under- or overestimation of the total population. The relationship between these partitions may also have implications for treatment, since nematicides may perform differently if nematodes are in the roots or if they are in the soil. This type of information may assist in refining the timing of nematicide applications and development of nematicide formulations.

**Root and rhizome weight.** Root and rhizome weight per standard core was less in field 2 than in field 1 during the 1988 growing season. This may have been a result of severe flaming of field 2 in the spring of 1988. However, P. penetrans populations per standard core were similar in fields 1 and 2 through late winter and spring of 1989, suggesting that flaming injury did not affect nematode population levels.

P. penetrans population. Except for the May 1988 P. penetrans peak in field 2, populations in fields 1 and 2 were lower during 1988 than in 1989 and early 1990. Although the severe winter of 1988-1989 may have kept the nematode populations at lower levels than in the following year, the abnormally cold weather (i.e., sustained temperatures below 0 C for over one week) may also have stressed the peppermint plants, making them more susceptible to parasitism. This could explain the higher densities observed during 1989. The milder winter of 1989-1990 appeared to have permitted greater survival into the spring of 1990.

P. penetrans exhibited patterns similar to those reported from peppermint in earlier studies (Pinkerton, 1983; Pinkerton and Jensen, 1983). The soil population peaked first in May, two weeks before root populations peaked, and secondly in mid-September. In the fall, soil populations peaked as nematodes migrated from senescent roots into soil. Root and soil populations were lowest in late winter. Soil populations peaked before those in roots in the Pinkerton and Jensen study, whereas in the present study, soil and root populations usually peaked simultaneously.

In other population studies, soil populations of P. penetrans on strawberries peaked in June, while root

populations peaked in July (DiEdwardo, 1961). Soil populations declined from July to September and were lowest in January, but root populations were stable from October through January and increased from March through July. P. penetrans populations under rye-tobacco rotation were generally low in summer and high in fall, but seasonal changes were not consistent (Olthof, 1971). Florini and Loria (1990) found that population increases of P. penetrans in pot studies were not related to root weight or to nematode densities in roots.

Some fluctuation in populations of the three nematode species monitored in the present study may represent generations. The life cycle duration of P. penetrans is 86 days at 15 C, 42-44 days at 20 C, and 30 to 31 days at 30 C (Maniyya, 1971). Times between population peaks in field 2 during the 1989 growing season were 56 days (May 4 to July 13), 40 days (July 13 to August 28) and 62 days (August 28 to November 16), all plausible egg-to-egg generation times. Kimpinski (1975) observed P. penetrans populations invading red clover roots by June 21 and increasing sharply in early August, again in mid-September, and in early November, coincident with production of four generations in roots. Gird (1977) observed a rapid increase in P. penetrans in potato roots in the first three weeks after planting. The population

declined and then increased again in week 5, 6, or 7. Each peak and following decline may have represented one generation.

Paratylenchus sp. populations. Populations of this species, like those of P. penetrans, were higher during 1989 and 1990 than during the previous growing season and winter. Again, although the severe winter may have repressed the winter nematode population, it may also have stressed the peppermint plants, making them better nematode hosts.

Unlike the other two fields, field 3 supported a much lower Paratylenchus sp. population during the 1989 growing season than during 1988. This field had extensive Verticillium wilt and was not vigorous during 1989. The field was disced in the fall of 1988 in an attempt to rejuvenate the stand by breaking up dense roots, but a mint rust outbreak in the early spring required propane flaming at the time of the usual spring flush of shoot growth. Between the resulting growth setback and the even more extensive wilt symptoms, peppermint growth was very poor during the 1989 growing season. Because the stand was so thin, there were few roots and thus fewer sites available for Paratylenchus sp. to parasitize. In addition, the mechanical action of tilling may have directly reduced the population. Despite the difference

in magnitude of population levels between the two years, populations appeared to be increasing towards the end of the 1989 season as observed in fields 1 and 2.

C. xenoplax populations. The population size of C. xenoplax in field 1 was comparatively low in winter, moderate during the growing season, and reached a peak in late summer during both yearly cycles of the study. The June 4, 1989 peak in field 1, when compared to the May 12 peak in the same field in 1988 suggests the possibility of a predictable spring population peak as well as one in the fall. The C. xenoplax population in field 2 was low during the 1988 growing season but began to increase in the fall of 1988. Population fluctuations in field 2 during 1988 paralleled those in field 1 in several instances, and an August 28, 1989 maximum of over 3000 per 500 g soil was nearly as high as the September 15 maximum in field 1. Although the cause of the C. xenoplax increase in field 2 is unknown, it may provide an example of a nematode population in the early stages of establishment as opposed to an established population repeating yearly patterns.

Some of the fluctuations in the C. xenoplax population may represent generation times. The life cycle of C. xenoplax takes 25 to 35 days (Seshadri, 1964, Jenkins and Taylor, 1967). Times between population peaks



in field 1 throughout the study period range from 41 to 61 days. Although these periods are somewhat longer than the documented generation time, they are shorter during summer than during winter and occur regularly, suggesting temperature-affected reproduction. The possibility of these peaks corresponding to successive generations of C. xenoplax needs further study.

P. penetrans, Paratylenchus sp., and C. xenoplax all exhibited a late August to mid-September population peak in both years (except for the nearly absent C. xenoplax in field 2 in 1988). These maxima follow the early August root weight maximum by three to five weeks. Strongly defined P. penetrans population peaks and vaguely discernable Paratylenchus sp. and C. xenoplax population peaks followed the spring root weight maximum by about the same time period. This time period approximates the generation time for these nematodes. The delay between root growth and nematode population growth may represent the time necessary for the development of a new generation.

### SUMMARY

The study of peppermint and its relationship to the three plant-parasitic nematodes P. penetrans, Paratylenchus sp., and C. xenoplax using the standard core approach has enabled comparison of root growth and nematode population dynamics through two yearly cycles of this perennial crop.

Peppermint root and rhizome weights reach maxima in early April and early August. P. penetrans populations follow a similar pattern, reaching maxima two to four weeks after root and rhizome weights. P. penetrans in the soil/root/rhizome system peaks in early May, declines during the growing season, peaks again in late summer or early fall, and declines to a lower level during the winter. Seventy to ninety percent of the population is in the roots in spring, 40-50% during the growing season, 20-40% at harvest, and 20-30% during the winter. Up to 20% of the population can be in the rhizomes in the spring, but rhizome population percentages are below 10% for the remainder of the year.

Paratylenchus sp. populations reach a low peak in June, but the yearly maximum is in August. The August peak follows a root weight peak by about three weeks. C. xenoplax populations fluctuate throughout the year but are

comparatively low in winter and higher in summer. The population peaks in August at the same time as the other two species.

Examination of the plant-nematode system using the standard core concept reinforces the importance of sampling both soil and roots when assessing endoparasitic nematodes. This approach facilitates measurement of the total nematode pressure on the plants by P. penetrans, an endoparasite in all components of the system (soil, roots, and rhizomes) as well as total pressure from the two additional ectoparasites. The information thus derived may assist in developing better sampling methods, in refining the timing of nematicide applications, and in the development of nematicide formulations.

THE EFFECT OF OXAMYL ON POPULATIONS OF  
PRATYLENCHUS PENETRANS, PARATYLENCHUS SP.,  
AND CRICONEMELLA XENOPLAX  
AND ON HAY AND OIL YIELD IN WESTERN OREGON PEPPERMINT.

Peppermint (Mentha piperita L.) is grown as a perennial no-till crop in Oregon. Hay is harvested in early August, and an essential oil is extracted by steam distillation. Pratylenchus penetrans is the dominant plant-parasitic nematode collected from peppermint in western Oregon (Pinkerton, 1983). The Paratylenchus sp. described earlier is often associated with P. penetrans and occasionally occurs as the only plant-parasitic nematode in peppermint stands. Criconemella xenoplax has been less commonly documented in nematode collections from peppermint. P. penetrans is a major component of peppermint stand decline in western Oregon (Pinkerton, 1983). Bergeson (1963) and Bergeson and Green (1979) observed from 34 to 40% reduction in foliage dry weight and from 70 to 80% reduction in root weight of M. piperita by P. penetrans. Peppermint stands parasitized by P. penetrans are characterized by shallow root systems and extensive bare patches. Paratylenchus hamatus reduced

yield in Mentha piperita, M. spicata, and M. cardiaca (Faulkner, 1963). Neither the effect of Paratylenchus sp. nor of C. xenoplax on M. piperita has been documented. The carbamate nematicide oxamyl has been used to decrease plant parasitic nematode densities and increase stand vigor in Oregon (Pinkerton, 1983). Effects of oxamyl on Paratylenchus sp. and C. xenoplax have not been documented. P. penetrans population decreases from oxamyl were evident after two seasons (Pinkerton et al., 1988). The present study compares P. penetrans, Paratylenchus sp., and C. xenoplax populations and peppermint hay weight, oil yield per kg hay, and oil yield/2 m<sup>2</sup> between oxamyl-treated and non-treated plots. In addition, two methods of measuring nematode populations over time are compared.

## MATERIALS AND METHODS

**Field description:** Field 1 soil, a silt loam, had 11% clay, 58% silt, 32% sand, and 0.1% coarse particles. This field had high Pratylenchus penetrans, Paratylenchus sp., and Criconebella xenoplax populations throughout the experiment. Field 2 soil, also a silt loam, had 8% clay, 65% silt, 27% sand, and <0.1% coarse particles. This field had high P. penetrans and Paratylenchus sp. populations and a steadily increasing C. xenoplax population. Both field 1 and 2 had pronounced weed problems, and field 1 had extensive patches of bare soil, which are typical symptoms of nematode-damaged peppermint. Field 2 was flamed more severely than usual in 1988 during this normal spring control measure for mint rust (Puccinia menthae Pers.) and probably contributed to low yield that year. Field 3 soil, another silt loam, had 17% clay, 75% silt, 8% sand, and 0.4% coarse particles. Fields 3 and 4 had a moderate Paratylenchus sp. population. All four fields had some bronzed, twisted leaves on stunted plants, symptoms of wilt caused by Verticillium dahliae Kleb., but damage was not extensive except in field 3. In addition, field 3 had been disced the previous fall, fragmenting the peppermint plants and decreasing stand vigor. A

severe mint rust outbreak in this field required spring flaming, normally omitted in new or recently disced fields.

**Plot Design:** Five pairs of plots (oxamyl-treated and non-treated) were established in each of the four peppermint fields. Fields 1, 2, and 4 were east of Monroe, Benton County, Oregon, and field 3 was north of Junction City, Lane County, Oregon. Location of each plot pair was selected at random and pairs were separated by at least 15.3 m in fields 1, 2, and 3. Treated and non-treated plots were in adjacent strips in field 4. Nematode numbers and hay and oil yields were measured in fields 1 and 2 during both 1988 and 1989, in field 3 during 1989 only, and in field 4 during 1988 only.

**Oxamyl application:** Oxamyl (1.1 kg a.i./ha) was applied to treated plots on May 26, 1988 to fields 1, 2, and 4, on April 24, 1989 to fields 1 and 2, and on April 27, 1989 to field 3. Non-treated plots received no oxamyl during the entire study period. In field 3, non-treated plots were tarped during application. In all other fields on all dates, non-treated plots were avoided by applicators during commercial field application. All fields received rain within 48 hours of application.

**Nematode sampling and assay:** In 1988, pretreatment samples were taken on April 8 in fields 1, 2, and 4;

posttreatment samples on June 9 in fields 1 and 2; and harvest samples on August 9 in all three fields. In 1989, pretreatment samples were taken on April 6 in fields 1, 2, and 3, posttreatment samples on May 18 in fields 1, 2, and 3, and harvest samples on August 8 in fields 1 and 2 and on July 24, 1989 in field 3.

Samples to determine population dynamics were collected in fields 1 and 2 at two week intervals during spring, summer, and fall and at monthly intervals during the winter from April 8, 1988 through March 28, 1990. Field 3 was sampled at two week intervals from April 6 through July 24, 1989.

Five cores approximately 15 cm deep and 7.5 cm in diameter were collected at random from each plot, were bulked into a single sample and sieved through 5 mm screen to separating mint roots and rhizomes from soil. The soil was mixed thoroughly, and nematodes were extracted from a 100 g sample using the wet sieving/sucrose centrifugation method described in chapter 1. A 20 g sample from each plot was oven dried for at least 8 hours and reweighed to determine soil moisture.

Roots and rhizomes from each plot were washed clean of soil and debris in a pressurized water stream from a hand held nozzle. Plant material was collected on a 6 mm screen, and roots were trimmed from rhizomes. After



decomposed portions were trimmed from rhizomes, the rhizomes were cut into approximately 5 mm segments. Root and rhizome samples from each plot were weighed and then placed in an intermittent misting chamber for seven days to extract endoparasitic nematodes.

Nematodes extracted from soil samples were left to settle in conical-bottomed centrifuge tubes for at least 24 hours, after which excess water was decanted and the nematodes and a small amount of water were poured into a counting dish. Between 50 and 100 nematodes of each of the three species studied were counted and multiplied by a conversion factor derived from the area of the dish counted to determine the number in the entire dish. Soil nematode counts were adjusted according to soil moisture to obtain nematodes per 100 g dry soil. Numbers of P. penetrans, Paratylenchus sp. , and C. xenoplax were converted to numbers of nematodes per standard core (as described in Chapter 2) consisting of 500 g dry soil and the peppermint roots and rhizomes included in that volume of soil. Nematodes of each species per 100 g dry soil were multiplied by five to obtain nematodes per 500 g dry soil. Fresh root weight and fresh rhizome weight per g dry soil collected were multiplied by 500 to obtain fresh root and rhizome weight per standard core for each plot. Numbers of P. penetrans/g fresh root or rhizome were

multiplied by these factors to obtain P. penetrans in roots and rhizomes per standard core for each plot. P. penetrans in roots, rhizomes, and soil per standard core were added to obtain total P. penetrans per standard core.

**Hay sampling and yield determination:** Peppermint hay was cut at ground level from a 2 m<sup>2</sup> area in the center of each plot in fields 1, 2, and 4 on August 9, 1988 and in fields 1, 2, and 3 on August 8, 1989. Fresh hay weight per plot was recorded immediately. Approximately 4.536 kg of fresh hay from each plot was retained in a burlap bag and air dried on a wire rack in the sun for one week.

Oil was distilled from dried hay samples from each plot using research scale oil distilling apparatus. Milliliters of oil distilled from each sample were recorded and converted to ml/kg fresh hay weight and to yield per 2 m<sup>2</sup> hay harvest area for each plot.

**Data analysis:** For each sampling date, P. penetrans in standard core soil, in standard core roots, in standard core rhizomes, and in total standard core were determined for each plot in fields 1, 2, and 3. Numbers of Paratylenchus sp. and C. xenoplax per standard core of 500 g dry soil were also determined for each plot on each sampling date.

Pretreatment, posttreatment, and harvest samples (point samples) of P. penetrans in standard core soil, in

standard core roots, and in standard core rhizomes, and total P. penetrans; Paratylenchus sp., and C. xenoplax per standard core were analyzed by treatment, date, and field with ANOVA. P. penetrans in standard core roots and C. xenoplax in the total standard core were analyzed separately by field due to a significant field x date interaction, and 2-way date x treatment ANOVA was performed for data in each field. P. penetrans in standard core rhizomes were separated by date and by field due to a three-way interaction, and thus student's t-test was used to compare treated and non-treated samples from each date in each field.

The area under the nematode population curves from treatment to harvest (April 8 to August 8, 1988 and from April 6 to August 9, 1989 in fields 1 and 2 and from April 6 to July 28, 1989 in field 3) were then determined for standard core soil, standard core roots, standard core rhizomes, and total standard core for each plot according to the summation of the expressions

$$(Y_2 - Y_1) * (X_2 - X_1) / 2,$$

where  $Y_2$  and  $Y_1$  are the nematode densities on a particular sampling date and on the date immediately preceeding it, respectively, and  $X_2$  and  $X_1$  are the two corresponding sampling dates (Shanner and Finney, 1977). The resulting summations from fields 1 and 2 were compared by analysis

of variance (ANOVA) by year and treatment. The summations from field 3 were compared using student's t-test.

AUNPCs of Paratylenchus sp. from treated and non-treated plots per standard core were compared using student's t-test. Pretreatment, posttreatment, and harvest samples from field 3 and pretreatment and harvest samples from field 4 were compared by date and treatment using ANOVA. Hay weight and the two oil yield measures from treated and non-treated plots in both field 3 and field 4 were compared within each field using student's t-test. AUNPCs and nematode numbers in pretreatment, posttreatment, and harvest samples were log transformed before analysis.

Hay weight ( $\text{kg}/2 \text{ m}^2$ ), oil yield ( $\text{ml}/\text{kg}$  fresh hay weight), and oil per  $2 \text{ m}^2$  plot were each compared by treatment and year for each field using ANOVA.

## RESULTS

Pretreatment, posttreatment, and harvest nematode sample analysis (Point Sample Analysis). Numbers of P. penetrans in standard core soil were significantly lower in treated plots than in nontreated plots in all but the 1988 post-treatment samples. (Table 6). P. penetrans in treated standard core roots were significantly lower in both field 1 and field 2 in posttreatment and harvest samples in both years. No significant treatment differences were observed between plots on other dates, and a significant date x treatment interaction was observed.

TABLE 6

PRATYLENCHUS PENETRANS IN STANDARD 500 g CORE SOIL<sup>1</sup>

## IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL

|                  | ----- 1988 <sup>2</sup> -----                  |                    |         | ----- 1989 <sup>3</sup> ----- |                    |         |
|------------------|--|--------------------|---------|-------------------------------|--------------------|---------|
|                  | Pre-<br>treatment                              | Post-<br>treatment | Harvest | Pre-<br>treatment             | Post-<br>treatment | Harvest |
| Non-<br>trt      | 137 <sup>5</sup> a <sup>6</sup> p <sup>7</sup> | 229aq              | 183apq  | 125ar                         | 282as              | 670as   |
| Trt <sup>4</sup> | 119bp  | 212ap              | 41bq    | 77br                          | 101bpr             | 229bs   |

PRATYLENCHUS PENETRANS IN STANDARD 500 g CORE ROOTS<sup>1</sup>

## IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL

|                  | ----- 1988 <sup>2</sup> -----                   |                    |         | ----- 1989 <sup>3</sup> ----- |                    |         |
|------------------|---|--------------------|---------|-------------------------------|--------------------|---------|
|                  | Pre-<br>treatment                               | Post-<br>treatment | Harvest | Pre-<br>treatment             | Post-<br>treatment | Harvest |
| Non-<br>Trt      | 1899 <sup>8</sup> a <sup>6</sup> p <sup>7</sup> | 363aq              | 1030ar  | 415aqs                        | 449ars             | 1165at  |
| Trt <sup>4</sup> | 1903ap  | 289bq              | 80br    | 443as                         | 231bt              | 483bt   |

<sup>1</sup>Data from fields 1 and 2 combined.<sup>2</sup>1988 sampling dates: pretreatment: April 8; posttreatment: June 9; harvest: August 9.<sup>3</sup>1989 sampling dates: pretreatment: April 6; posttreatment: May 18; harvest: August 8.<sup>4</sup>Plots treated with oxamyl (1.1 a.i./ha) on May 26, 1988 and on April 24, 1989.<sup>5</sup>Mean P. penetrans in soil component of standard 500 g core.  
n = 10.<sup>6</sup>Means in the same column followed by the same letter (a,b) are not significantly different (p = 0.01) according to Fisher's Protected LSD Procedure.<sup>7</sup>Means in the same row followed by the same letter (p,q,r) are not significantly different (p = 0.01) according to Fisher's Protected LSD Procedure.<sup>8</sup>Mean P. penetrans in root component of standard 500 g core.  
n = 10.<sup>9</sup>High tide at Monroe, Oregon on Jan 5, 1990 was 9.0 ft (2.7 m) at 6:13 AM PST.

P. penetrans in standard core rhizomes from treated plots were significantly lower in field 1 at 1988 harvest and in field 2 at 1989 harvest (Table 7). No other significant treatment differences were observed on other dates. Total P. penetrans per standard core was significantly lower in treated than in non-treated plots at 1988 harvest and in 1989 posttreatment and harvest samples (Table 8). Significant date x treatment interaction was observed.

TABLE 7

PRATYLENCHUS PENETRANS IN STANDARD 500 g CORE RHIZOMES

## IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL

| ----- 1988 <sup>1</sup> ----- |                   |                    | ----- 1989 <sup>2</sup> ----- |                   |                    |         |
|-------------------------------|-------------------|--------------------|-------------------------------|-------------------|--------------------|---------|
|                               | Pre-<br>treatment | Post-<br>treatment | Harvest                       | Pre-<br>treatment | Post-<br>treatment | Harvest |
| FIELD 1                       |                   |                    |                               |                   |                    |         |
| Non-<br>Trt                   | 0.4 <sup>3</sup>  | 5.8                | 79.6                          | 1.0               | 5.0                | 20.2    |
| Trt <sup>4</sup>              | 0.2               | 31.2               | 0.8* <sup>5</sup>             | 45.9              | 12.0               | 5.6     |
| FIELD 2                       |                   |                    |                               |                   |                    |         |
| Non-<br>Trt                   | 0.2               | 21.4               | 7.0                           | 27.8              | 93.0               | 354.6   |
| Trt                           | 8.8               | 9.2                | 0.2                           | 19.4              | 14.4               | 8.2*    |

<sup>1</sup>1988 sampling dates: pretreatment: April 8; posttreatment: June 9; harvest: August 9.

<sup>2</sup>1989 sampling dates: pretreatment: April 6; posttreatment: May 18; harvest: August 8.

<sup>3</sup>Mean P. penetrans in rhizome component of 500 g standard core.  
n = 5.

<sup>4</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988 and on April 24, 1989.

<sup>5</sup>Treated means followed by \* differed significantly (p = 0.01) from nontreated means according to Student's t-test.



TABLE 8  
PRATYLENCHUS PENETRANS IN STANDARD 500 g CORE:<sup>1</sup>  
 SOIL, ROOTS, AND RHIZOME COMPONENTS COMBINED  
 IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL

|                  | ----- 1988 <sup>2</sup> -----      |                    |         | ----- 1989 <sup>3</sup> ----- |                    |         |
|------------------|------------------------------------|--------------------|---------|-------------------------------|--------------------|---------|
|                  | Pre-<br>treatment                  | Post-<br>treatment | Harvest | Pre-<br>treatment             | Post-<br>treatment | Harvest |
| Non-<br>Trt      | 2037 <sup>a</sup> 6 <sup>p</sup> 7 | 606aq              | 1256ar  | 526as                         | 780at              | 2023ap  |
| Trt <sup>4</sup> | 2027ap                             | 512aq              | 121br   | 555as                         | 345bq              | 719bs   |

<sup>1</sup>Data for fields 1 and 2 combined.

<sup>2</sup>1988 sampling dates: pretreatment: April 8; posttreatment: June 9; harvest: August 9.

<sup>3</sup>1989 sampling dates: pretreatment: April 6; posttreatment: May 18; harvest: August 8.

<sup>4</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988 and on April 24, 1989.

<sup>5</sup>Mean *P. penetrans* in soil, root, and rhizome components of standard 500 g core. n = 10.

<sup>6</sup>Means in the same column followed by the same letter (a,b) are not significantly different (p = 0.01) according to Fisher's Protected LSD Procedure.

<sup>7</sup>Means in the same row followed by the same letter (p,q) are not significantly different (p = 0.01) according to Fisher's Protected LSD Procedure.

No significant differences were observed between treatments for Paratylenchus sp. treatments in fields 1 or 2, but populations in each field increased significantly between posttreatment and harvest samples (Table 9). In field 1, no significant treatment or date effects were observed C. xenoplax populations, while in field 2, significant differences in this species were observed between 1988 and 1989 densities (Table 10). No differences between treatments for C. xenoplax were observed in this field.

**AUNPC Analysis.** No significant differences between treated and non-treated plots were observed in analysis of AUNPCs of P. penetrans in the soil component of a standard core in either field 1 or field 2 (Table 11). No significant treatment differences were observed in AUNPCs of P. penetrans in field 1 standard core roots. AUNPCs of P. penetrans in field 2 standard core roots were significantly smaller in 1988 in treated than in non-treated plots, but there was no treatment effect in 1989. In field 1, no significant differences between standard core rhizome P. penetrans AUNPCs of treated and non-treated plots were observed in 1988, while in 1989, rhizome P. penetrans AUNPCs from treated plots were significantly larger than in non-treated plots (Table 12). No significant treatment or year differences were detected

TABLE 9

PARATYLENCHUS SP. IN STANDARD 500 g CORE SOIL<sup>1</sup>

IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL

|                  | ----- 1988 <sup>2</sup> -----                  |                    |         | ----- 1989 <sup>3</sup> ----- |                    |         |
|------------------|--|--------------------|---------|-------------------------------|--------------------|---------|
|                  | Pre-<br>treatment                              | Post-<br>treatment | Harvest | Pre-<br>treatment             | Post-<br>treatment | Harvest |
| Non-<br>trt      | 410 <sup>5</sup> a <sup>6</sup> p <sup>7</sup> | 1607aq             | 2067aq  | 376ap                         | 882aq              | 7307ar  |
| Trt <sup>4</sup> | 552ap  | 975ap              | 2315aq  | 593ap                         | 721ap              | 2361aq  |

<sup>1</sup>Data for fields 1 and 2 combined.<sup>2</sup>1988 sampling dates: pretreatment: April 8; posttreatment: June 9; harvest: August 9.<sup>3</sup>1989 sampling dates: pretreatment: April 6; posttreatment: may 18; harvest: August 8.<sup>4</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988 and on April 24, 1989.<sup>5</sup>Mean Paratylenchus sp. in 500 g standard core soil. n = 10.<sup>6</sup>Means in the same column followed by the same letter (a,b) are not significantly different (p = 0.01) according to Fisher's Protected LSD Procedure.<sup>7</sup>Means in the same row followed by the same letter (p,q) are not significantly different (p = 0.01) according to Fisher's Protected LSD Procedure.

TABLE 10

CRICONEMELLA XENOPLAX IN STANDARD 500 g CORE SOIL  
IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL

| ----- 1988 <sup>1</sup> ----- |  |                    | ----- 1989 <sup>2</sup> ----- |                   |                    |         |
|-------------------------------|--|--------------------|-------------------------------|-------------------|--------------------|---------|
|                               | Pre-<br>treatment                              | Post-<br>treatment | Harvest                       | Pre-<br>treatment | Post-<br>treatment | Harvest |
| FIELD 1                       |  |                    |                               |                   |                    |         |
| Non-<br>Trt                   | 239 <sup>4</sup> a <sup>5</sup> p <sup>6</sup> | 319ap              | 1533ap                        | 584ap             | 974ap              | 1196ap  |
| Trt <sup>3</sup>              | 256ap  | 275ap              | 914ap                         | 832ap             | 1762ap             | 1281ap  |
| FIELD 2                       |  |                    |                               |                   |                    |         |
| Non-<br>Trt                   | 3ap  | 17agr              | 56ar                          | 383as             | 456at              | 1687au  |
| Trt                           | 0ap  | 8ap                | 0ap                           | 283aq             | 294aq              | 267aq   |

<sup>1</sup>1988 sampling dates: pretreatment: April 8; posttreatment: June 9; harvest: August 9.

<sup>2</sup>1989 sampling dates: pretreatment: April 6; posttreatment: May 18; harvest: August 8.

<sup>3</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988 and on April 24, 1989.

<sup>4</sup>Mean C. xenoplax in soil component of standard 500 g soil. n = 5.

<sup>5</sup>Means in the same column in the same field followed by the same letter are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

<sup>6</sup>Means in the same row followed by the same letter (p,q,r) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

TABLE 11  
 AREA UNDER NEMATODE POPULATION CURVE  
PRATYLENCHUS PENETRANS IN STANDARD 500 g CORE

## FIELD 1

|      | Soil  |                      | Roots       |         |
|------|---|----------------------|-------------|---------|
|      | Non-treated                                     | Treated <sup>1</sup> | Non-treated | Treated |
| 1988 | 1845 <sup>2</sup> a <sup>3</sup> p <sup>4</sup> | 1211ap               | 7976ap      | 7667ap  |
| 1989 | 2232ap  | 1171ap               | 4190bp      | 2896bp  |

## FIELD 2

|      | Soil        |         | Roots       |         |
|------|-------------|---------|-------------|---------|
|      | Non-treated | Treated | Non-treated | Treated |
| 1988 | 2386ap      | 1757ap  | 11459ap     | 9070aq  |
| 1989 | 2809ap      | 1463ap  | 9039bp      | 4232ap  |

<sup>1</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988 and on April 24, 1989.

<sup>2</sup>Area under nematode population curve from early April to early August (n = 5).

<sup>3</sup>Means in the same column in the same field followed by the same letter (a,b) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

<sup>4</sup>Means in the same row within the same core component followed by the same letter (p,q) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

TABLE 12  
 AREA UNDER NEMATODE POPULATION CURVE  
PRATYLENCHUS PENETRANS IN STANDARD 500 g CORE  
 FIELD 1

|      | <u>Rhizomes</u>                                |                            | <u>Total core</u>  |                |
|------|--|----------------------------|--------------------|----------------|
|      | <u>Non-treated</u>                             | <u>Treated<sup>1</sup></u> | <u>Non-treated</u> | <u>Treated</u> |
| 1988 | 509 <sup>2</sup> a <sup>3</sup> p <sup>4</sup> | 831ap                      | 11023ap            | 10043ap        |
| 1989 | 79bp   | 140bq                      | 6663bp             | 4472bq         |

FIELD 2

|      | <u>Rhizomes</u>    |                | <u>Total Core</u>  |                |
|------|--------------------|----------------|--------------------|----------------|
|      | <u>Non-treated</u> | <u>Treated</u> | <u>Non-treated</u> | <u>Treated</u> |
| 1988 | 1608ap             | 823ap          | 15454ap            | 11862aq        |
| 1989 | 1038ap             | 257ap          | 12671ap            | 5953aq         |

<sup>1</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988 and on April 24, 1989.

<sup>2</sup>Area under nematode population curve from early April to early August (n = 5).

<sup>3</sup>Means in the same column in the same field followed by the same letter (a,b) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

<sup>4</sup>Means in the same row within the same core component followed by the same letter (p,q) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

in field 2 standard core rhizome P. penetrans AUNPCs. P. penetrans total standard core densities were significantly lower in field 1 in 1989 and in field 2 in both years.

No significant differences in Paratylenchus sp. AUNPCs between treated and non-treated plots were observed in either field 1 or field 2 during 1988 or 1989 (Table 13). C. xenoplax densities were significantly higher in non-treated in treated plots in field 2 in 1988, but no significant differences were observed between treatments in field 2 in 1989 or in field 1 during either year (Table 13). In field 2, the 1989 AUNPC was significantly larger in 1989 than in 1988, while in field 1, there was no significant difference between years.

TABLE 13  
AREA UNDER NEMATODE POPULATION CURVE  
NEMATODES IN STANDARD 500 g CORE  
FIELD 1

|      | <u>Paratylenchus sp.</u>                          |                      | <u>C. xenoplax</u> |          |
|------|---|----------------------|--------------------|----------|
|      | Non-treated                                       | Treated <sup>1</sup> | Non-treated        | Treated  |
| 1988 | 15,495 <sup>2</sup> a <sup>3</sup> p <sup>4</sup> | 14,988ap             | 8,477ap            | 13,828ap |
| 1989 | 27,836ap  | 9,275ap              | 9,318ap            | 13,613ap |

FIELD 2

|      | <u>Paratylenchus sp.</u> |          | <u>C. xenoplax</u> |         |
|------|--------------------------|----------|--------------------|---------|
|      | Non-treated              | Treated  | Non-treated        | Treated |
| 1988 | 6,911ap                  | 12,689ap | 373ap              | 42aq    |
| 1989 | 14,509ap                 | 11,123ap | 8,660bp            | 2,843bp |

<sup>1</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988, and on April 24, 1990.

<sup>2</sup>Area under nematode population curve from early April to early August (n = 5).

<sup>3</sup>Means in the same column in the same field followed by the same letter (a,b) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

<sup>4</sup>Means in the same row within the same core component followed by the same letter (p,q) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).



**Population Dynamics:** P. penetrans per standard core population curves from non-treated plots resemble those from treated plots, but at most points, nematode numbers in non-treated plots were higher, especially at population peaks. (Figures 25 and 26). Population curves of Paratylenchus sp. per standard core from treated and non-treated plots also resembled each other, but the nematode numbers in treated plots were sometimes higher than those in non-treated plots (Figures 27 and 28). In field 1, population curves of C. xenoplax per standard core in treated and non-treated plots also increased and decreased simultaneously, and nematode numbers from oxamyl-treated plots were often higher than those from non-treated plots (Figure 29). In field 2, C. xenoplax densities were almost always lower in treated plots (Figure 30).

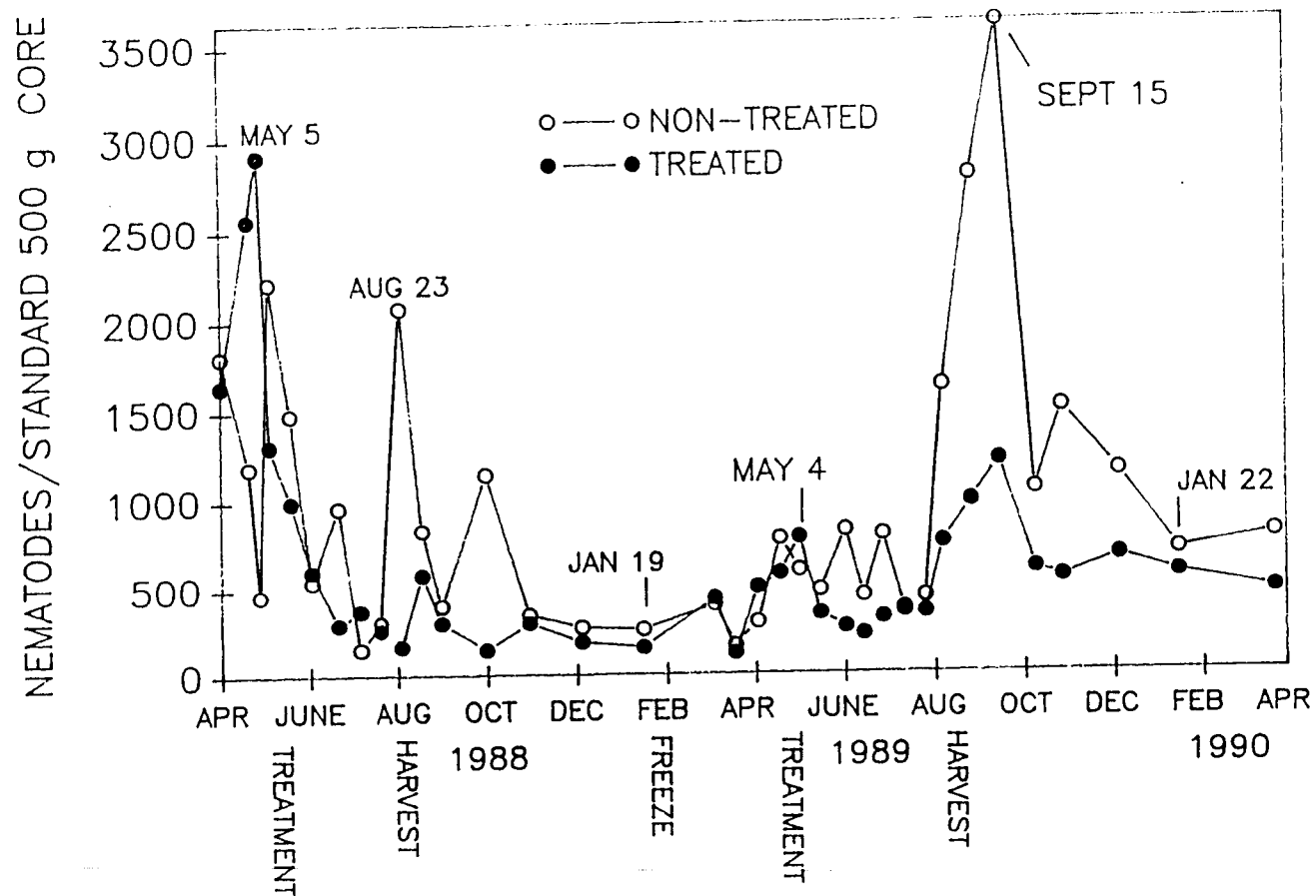


FIGURE 25. PRATYLENCHUS PENETRANS IN A STANDARD CORE, FIELD 1

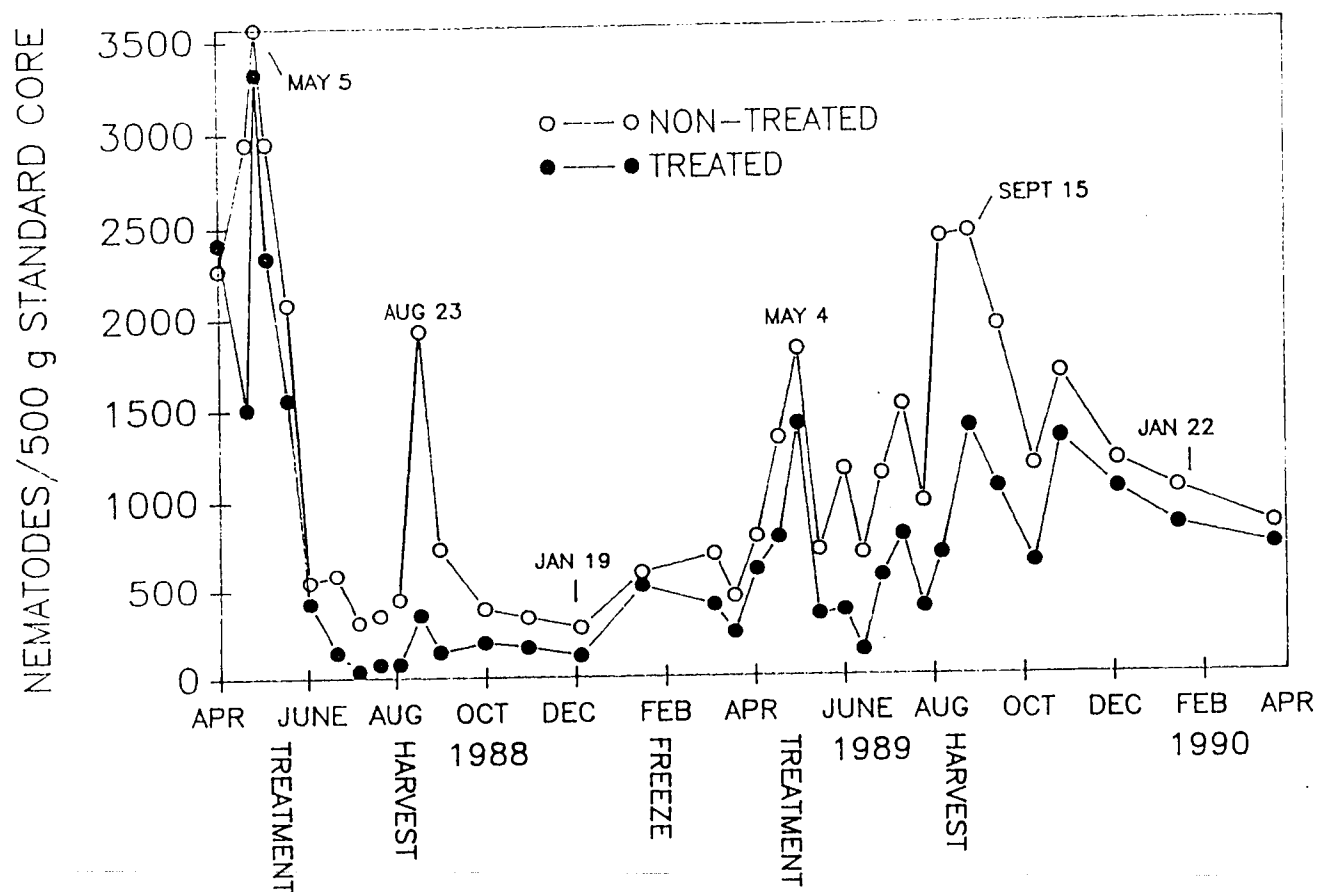


FIGURE 26. PRATYLENCHUS PENETRANS IN A STANDARD CORE, FIELD 2

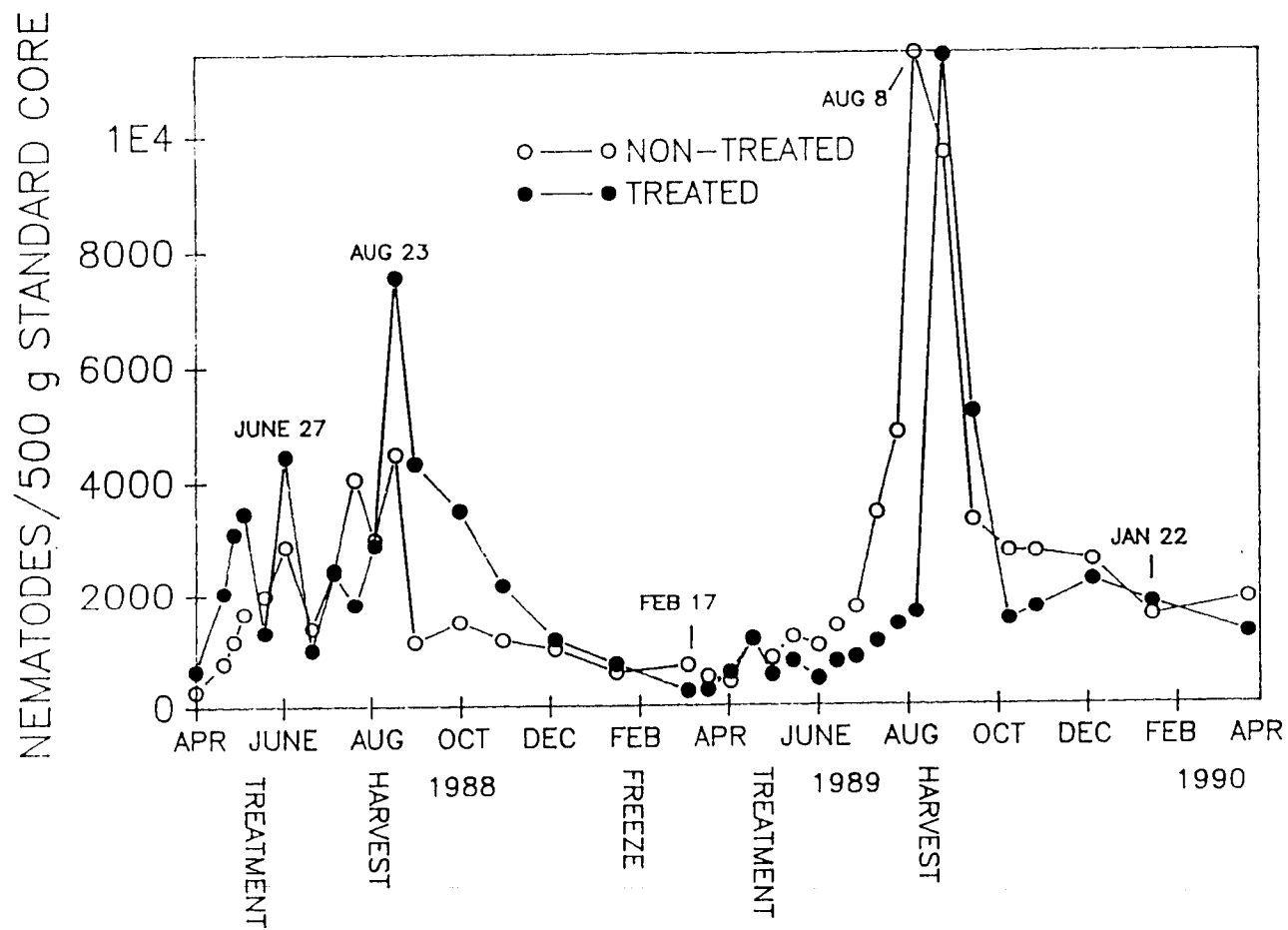


FIGURE 27. PARATYLENCHUS SP. IN A STANDARD CORE, FIELD 1

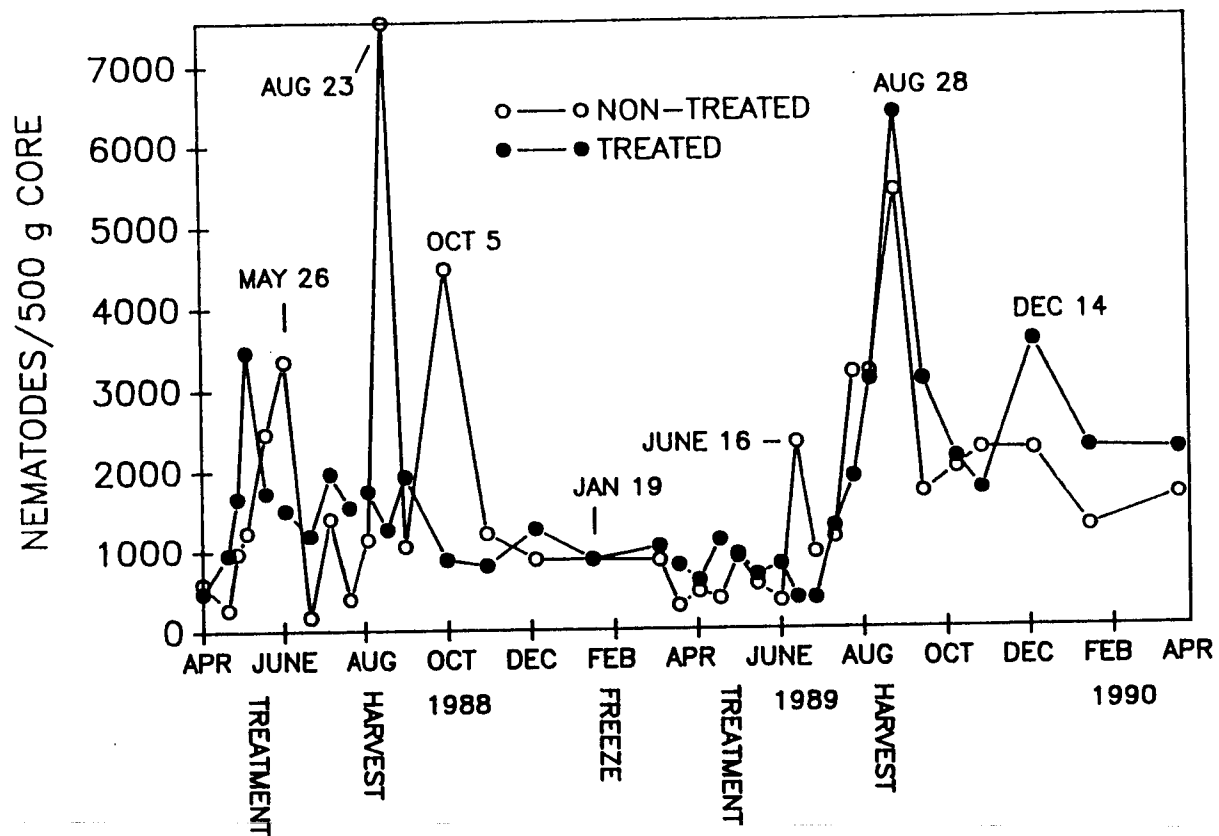


FIGURE 28. PARATYLENCHUS SP. IN A STANDARD CORE, FIELD 2

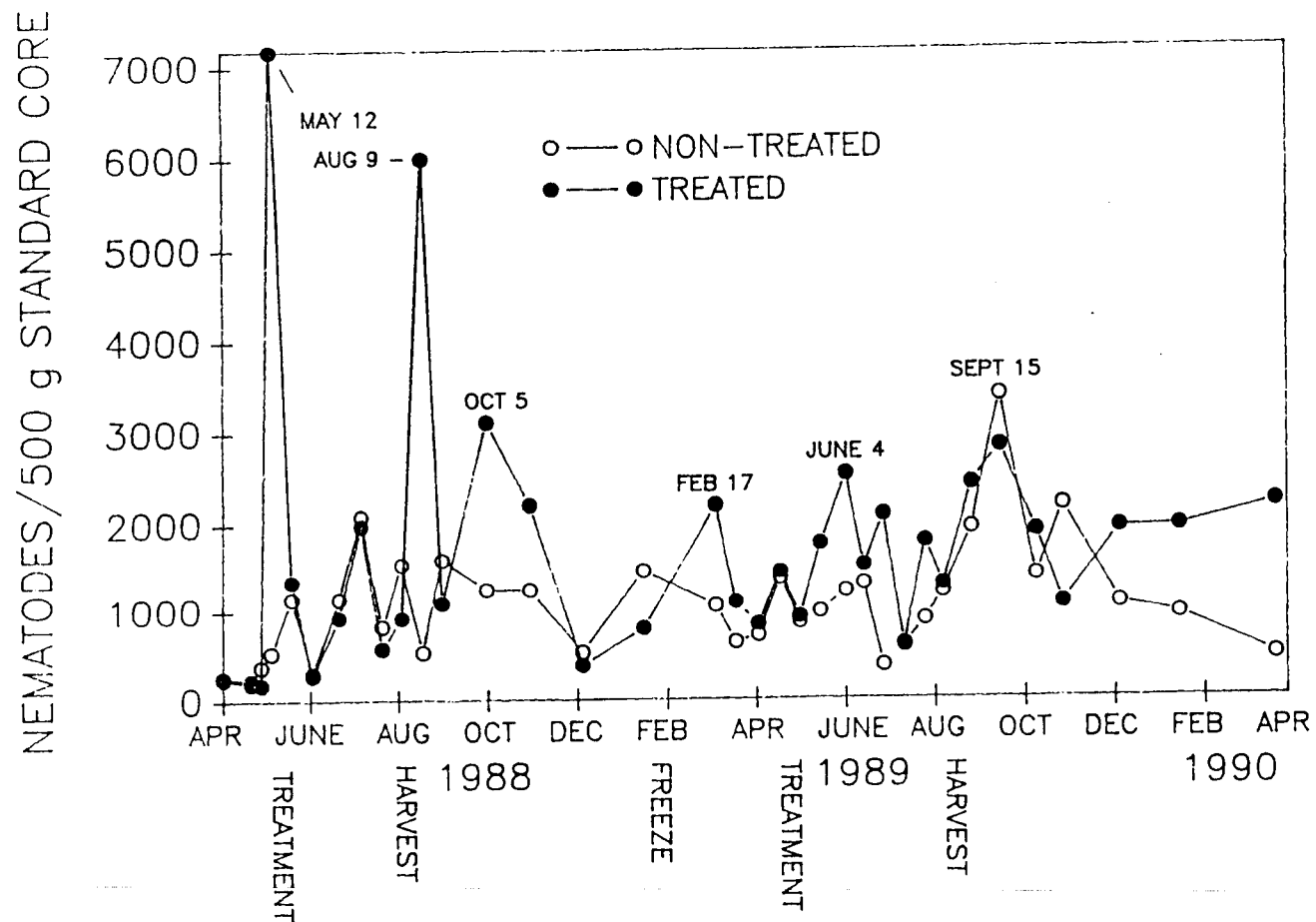


FIGURE 29. CRICONEMELLA XENOPLAX IN A STANDARD CORE, FIELD 1

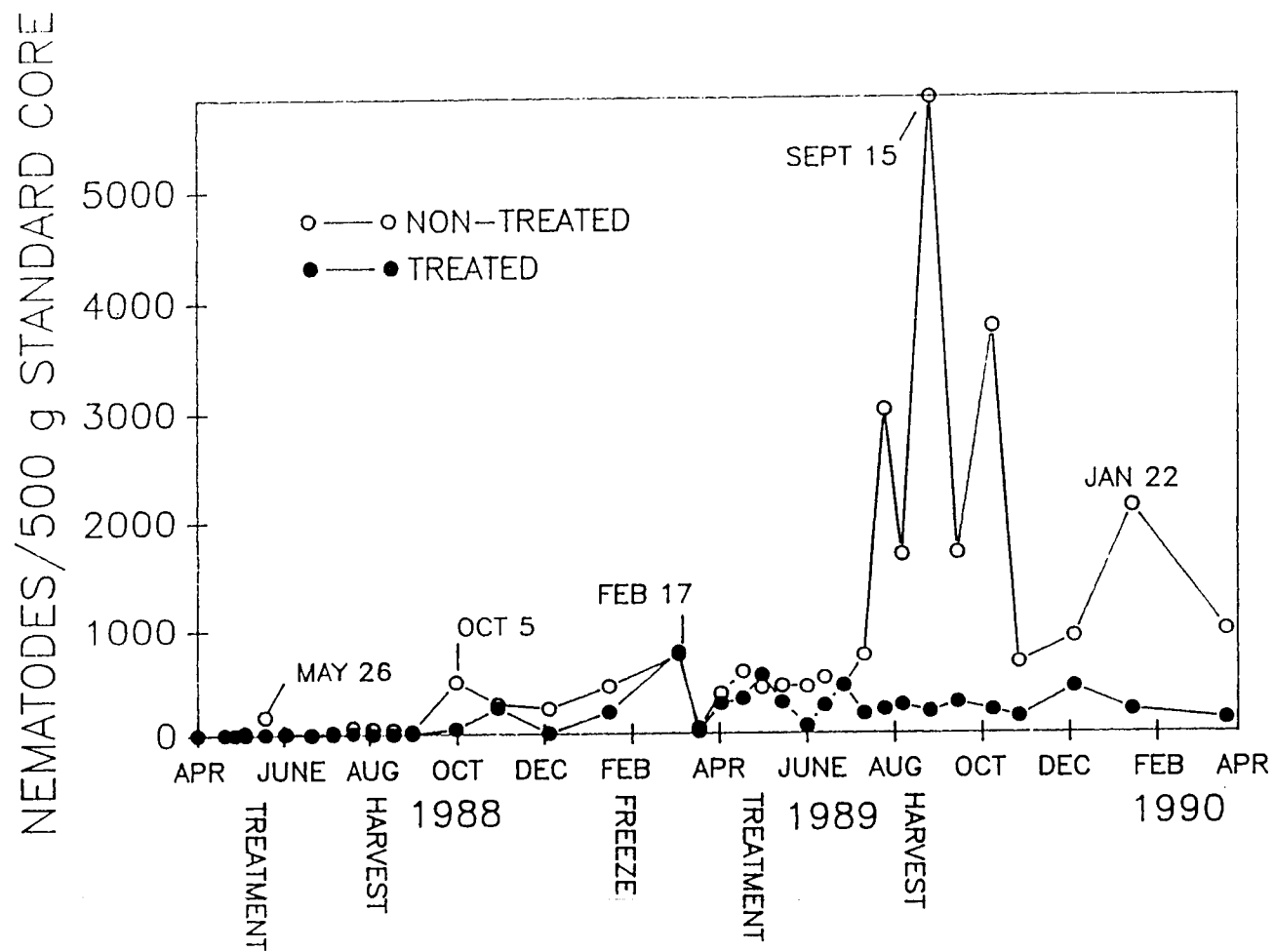


FIGURE 30. CRICONEMELLA XENOPLAX IN A STANDARD CORE, FIELD 2

The populations in treated plots remained relatively low throughout the study period, while those in nontreated plots increased dramatically during the 1989 growing season.

**Analysis of yield measurements.** No significant treatment or field differences were detected in kg fresh hay/2 m<sup>2</sup> in 1988 (Table 14). In 1989, treated plots in field 1 produced significantly more hay than non-treated plots, but treatments in field 2 exhibited no significant difference. No significant differences between treatments were observed in mint oil production (ml/kg fresh hay) from either field in 1988, while in 1989, oil production from treated plots was significantly lower than that from non-treated plots in both fields. Field 2 oil production was significantly higher than that from field 1 in 1988, but there were no significant differences between fields in 1989. No significant treatment or field differences in ml mint oil/2 m<sup>2</sup> field area were detected between treated and non-treated plots.



TABLE 14  
HAY WEIGHT AND OIL YIELD  
FIELDS 1 AND 2

|                  | Hay:<br>kg/2 m <sup>2</sup>                     |                  | Oil:<br>ml/kg fresh hay |        | Oil:<br>ml/2 m <sup>2</sup> |         |
|------------------|---|------------------|-------------------------|--------|-----------------------------|---------|
|                  | Non-<br>trt                                     | Trt <sup>1</sup> | Non-<br>trt             | Trt    | Non-<br>trt                 | Trt     |
| Field 1,<br>1988 | 3.50 <sup>2</sup> a <sup>3</sup> p <sup>4</sup> | 4.89ap           | 3.17ap                  | 2.97ap | 11.28ap                     | 14.56ap |
| Field 2,<br>1988 | 4.82ap  | 4.23ap           | 3.39bp                  | 3.65bp | 15.82ap                     | 15.25ap |
| Field 1,<br>1989 | 2.46ap  | 4.38aq           | 3.67ap                  | 2.98aq | 7.74ap                      | 12.33ap |
| Field 2,<br>1989 | 4.14bp  | 4.92ap           | 3.29ap                  | 2.79aq | 11.97ap                     | 12.62ap |

<sup>1</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988, and on April 24, 1989.

<sup>2</sup>n = 5.

<sup>3</sup>Means in the same column followed by the same letter (a,b) are not significantly different (p = 0.05) according to Fisher's Protected LSD Procedure.

<sup>4</sup>Means in the same row within the same yield measurement followed by the same letter (p,q) are not significantly different (p = 0.05) according to Fisher's Protected LSD Procedure.

**Paratylenchus sp. studies in Fields 3 and 4.**

Posttreatment and harvest densities of Paratylenchus sp. from treated plots were significantly lower than those from nontreated plots in field 3 (Table 15). The AUNPC of the treated plots was significantly smaller than that of the non-treated plots (Table 15, Figure 31). No treatment differences were observed in hay or oil yield.

In field 4, Paratylenchus sp. levels were significantly higher in harvest samples than in pre-treatment samples, but there was no significant treatment effect (Table 16). Significantly more hay was harvested from treated than from non-treated plots in field 4, but oil measured in both ml/kg fresh hay weight and ml/2 m<sup>2</sup> area exhibited no significant differences.

TABLE 15  
PARATYLENCHUS SP. IN STANDARD 500 g CORE SOIL  
 IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL  
 FIELD 3

|                      | Pre-treatment <sup>1</sup>                     | Post-treatment <sup>2</sup> | Harvest <sup>3</sup> |
|----------------------|--|-----------------------------|----------------------|
| Non-treated          | 493 <sup>5</sup> a <sup>6</sup> p <sup>7</sup> | 432ap                       | 2092ap               |
| Treated <sup>4</sup> | 516ap  | 292bp                       | 226bp                |

Hay weight and oil yield, 1989

|             | Hay:<br>kg/2 m <sup>2</sup> | Oil:<br>ml/kg | Oil:<br>ml/2 m <sup>2</sup> |
|-------------|-----------------------------|---------------|-----------------------------|
| Non-treated | 2.36a                       | 2.70a         | 6.98a                       |
| Treated     | 2.81a                       | 2.79a         | 8.48a                       |

Area Under Paratylenchus sp. population curve

|             |                     |
|-------------|---------------------|
| Non-treated | 74,044              |
| Treated     | 32,548 <sup>*</sup> |

<sup>1</sup>Pretreatment sample date: April 6, 1989.

<sup>2</sup>Posttreatment sample date: May 18, 1989.

<sup>3</sup>Harvest sample date: July 24, 1989.

<sup>4</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on April 27, 1989.

<sup>5</sup>n = 5.

<sup>6</sup>Means in the same column followed by the same letter (a,g) are not significantly different according to Fisher's protected LSD Procedure (p = 0.01).

<sup>7</sup>Means in the same row followed by the same letter (p,q) are not significantly different according to Fisher's Protected LSD Procedure (p = 0.01).

<sup>\*</sup>Mean is significantly higher (p = 0.01) from non-treated mean.

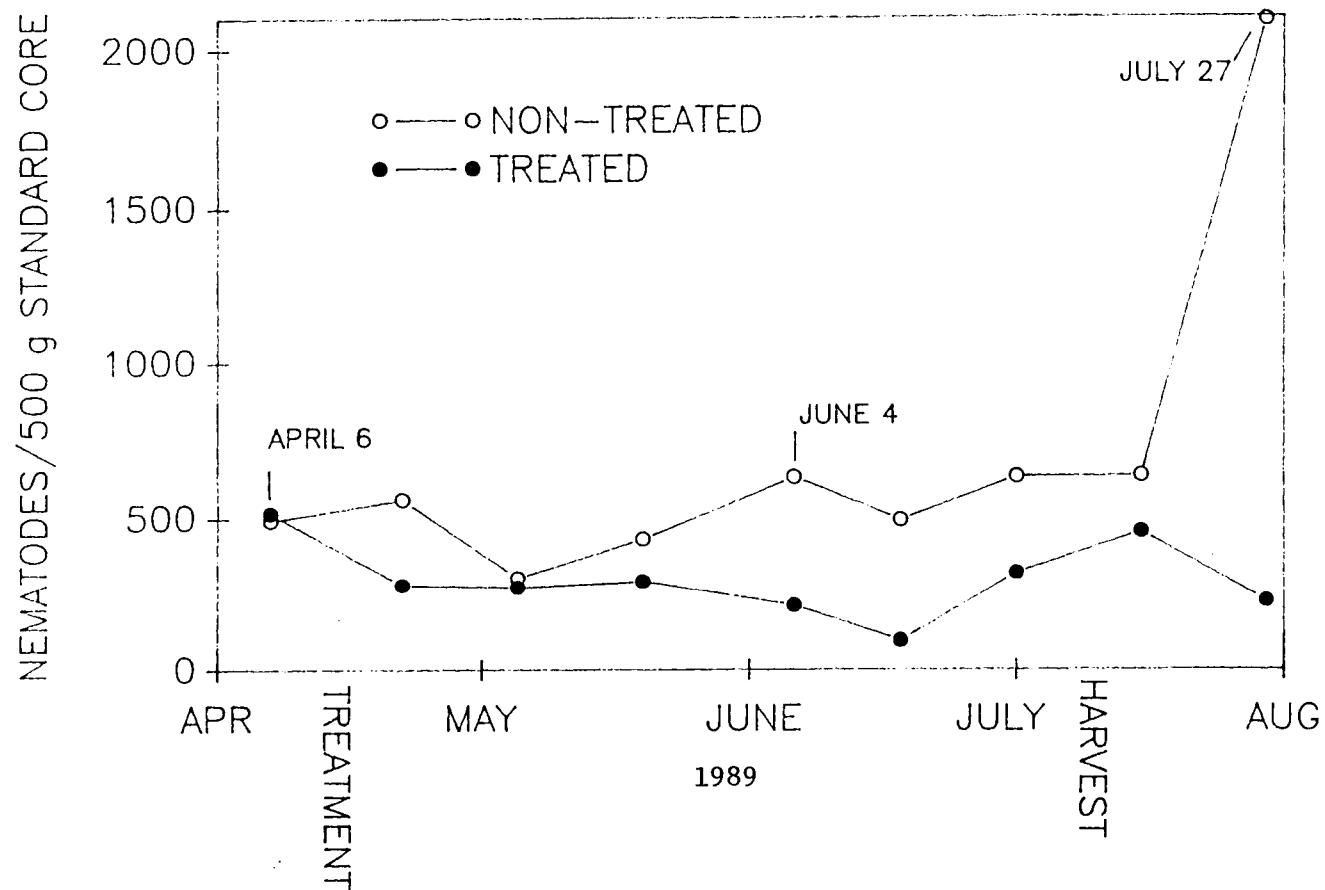


FIGURE 31. PARATYLENCHUS SP. IN A STANDARD CORE, FIELD 3

TABLE 16  
PARATYLENCHUS SP. IN STANDARD 500 g CORE SOIL  
IN FIELD PLOTS TREATED OR NOT TREATED WITH OXAMYL  
 FIELD 4

|                      | Pre-treatment <sup>1</sup>                     | Harvest <sup>2</sup> |
|----------------------|--|----------------------|
| Non-treated          | 930 <sup>4</sup> a <sup>5</sup> p <sup>6</sup> | 2498aq               |
| Treated <sup>3</sup> | 534ap  | 2167aq               |

Hay weight and oil yield, 1988

|             | Hay:<br>kg/2m <sup>2</sup> | Oil:<br>ml/kg | Oil:<br>ml/2m <sup>2</sup> |
|-------------|----------------------------|---------------|----------------------------|
| Non-treated | 6.01 <sup>4</sup> a        | 2.71a         | 16.28a                     |
| Treated     | 8.14b                      | 2.23a         | 18.15a                     |

<sup>1</sup>Pretreatment sampling date: April 8, 1988.

<sup>2</sup>Harvest sampling date: August 9, 1988.

<sup>3</sup>Plots treated with oxamyl (1.1 kg a.i./ha) on May 26, 1988.

<sup>4</sup>Mean Paratylenchus sp. in soil component of standard 500 g core.  
n = 5.

<sup>5</sup>Means in the same column followed by the same letter (a,b) are not different according to Fischer's protected LSD Procedure (p = 0.01).

<sup>6</sup>Means in the same row followed by the same letter (p,q) are not different according to Fischer's protected LSD Procedure (p = 0.01).

## DISCUSSION

Analysis of pretreatment, posttreatment, and harvest nematode populations detected more significant differences between treatments and thus appears to be more sensitive than the AUNPC population analysis. While analysis of the area under P. penetrans population curves from the different standard core components provides an integrated interpretation of relative population sizes in treated and non-treated plots over time, fewer treatment differences were detected using this method. Thus, the AUNPC analysis correlates better with the yield analyses, since no significant differences were observed in oil produced/2 m<sup>2</sup> area. P. penetrans in standard core soil, roots, and rhizomes exhibited few treatment differences compared to those detected in point samples at pretreatment, posttreatment, and harvest samples. Treatment differences between pretreatment populations were detected in the point sample analysis, indicating either uneven P. penetrans population distribution or overly sensitive analysis. Similarly, more significant differences in Paratylenchus sp. and C. xenoplax were detected in the point sample than in the AUNPC analysis.

Oxamyl appeared to be effective in suppressing *P. penetrans* populations based on analysis of standard core soil and root population components and therefore the total standard core population. Pinkerton et al. (1988) found that yield response to oxamyl application was not correlated with *P. penetrans* population reductions at midseason or harvest. In the present study, *P. penetrans* populations were significantly lower in posttreatment samples, approximately one month after oxamyl application. Oxamyl suppression of rhizome populations appeared limited, but this may be due to inherently low populations usually present in rhizomes. This small population component may not be an accurate indication of the response of the population as a whole.

*Paratylenchus* sp. populations in fields 1 and 2 were not suppressed by oxamyl. At times, populations of this species were higher in treated than in non-treated plots. Pinkerton (1983) observed that where *P. penetrans* populations decreased, populations of *Paratylenchus* sp. increased, possibly as a result of decreased competition from *P. penetrans*. In contrast, *Paratylenchus* sp. populations in field 3 treated plots were lower than those in non-treated plots. In this field, *Paratylenchus* sp. forms a nearly monospecific nematode population. Competition from other plant-parasitic species may

confound the effect of oxamyl in fields with multispecific nematode populations, but with that factor missing, oxamyl may be more effective. In contrast, however, oxamyl had no significant effect on the monospecific Paratylenchus sp. population in field 4.

No significant effect of oxamyl treatments on C. xenoplax was detected. However, in field 2, the population of this species, which was very low in 1988, increased dramatically in nontreated plots but not in treated plots in 1989.

Oxamyl treatments were applied when the P. penetrans population was approaching its spring peak. After the normal population decline following this peak, nematode numbers in oxamyl-treated plots plateaued, while numbers in non-treated plots increased to a late summer peak as observed in other peppermint studies (Pinkerton 1983; See Chapter 2). This is well illustrated by comparison of total standard cores and root populations in treated and non-treated plots. Paratylenchus sp. in field 3 responded similarly, with populations in treated plots remaining lower than those in non-treated plots during normal population peaks.

Lower root densities of P. penetrans in treated plots from two to seven weeks after oxamyl application are indicative of the systemic nature of this chemical, since



uptake by the plant increases its longevity in the plant-soil system. Lower root populations of P. penetrans from treated plots in fields 1 and 2 in 1989 pre-treatment samples suggest that suppression of populations by oxamyl remained for months after the 1988 spring application. The half life of oxamyl is 1 week, and only 5 % of the compound remains after 30 days (Bunt and Noordink, 1977, in Pinkerton et al., 1988). At rates registered for use on peppermint, its action appears mainly prophylactic rather than therapeutic (Pinkerton et al., 1988). If young roots are protected from spring nematode invasion, strong root systems develop, and the resulting vigorous plants tolerate nematode penetration and feeding with little yield loss. As in this study, oxamyl treatment effects on nematode populations in the Pinkerton et al. study were still evident during the growing season after application.

Plots with more nematodes, whether as a result of oxamyl treatment or to lower initial field populations, produced less hay. This may have been due to the effect of the nematodes on the plants, or to a direct growth response by the plants to oxamyl, or to a combination of these factors. Yield response to oxamyl in field 4, a healthy field with a low Paratylenchus sp. population, suggests such promotion. Although a moderate population of Paratylenchus sp. was detected in field 4, it was used

to test for growth promotion by oxamyl, since it is a healthy, high-producing peppermint field. Influences from this nematode population are assumed to be minimal, (R. E. Ingham, Personal Communication) and the significantly higher hay weight in treated plots is attributed to the growth promotion of oxamyl. More hay was produced in treated than in non-treated plots. However, more oil per kilogram fresh hay was produced in non-treated than in treated plots, leading to a lack of significant difference between treatments in the oil per unit field area produced. The difference in oil/kg fresh hay between fields 4 and fields 1 and 2 may be a result of early morning harvesting and weighing of mint while field 4 hay was still wet from dew. The significant difference between oil yield in 1988 and 1989 may be a result of a different still operator or to weather differences during the growing seasons.

Paratylenchus sp. densities on peppermint increased from spring through summer, peaking in August. Populations in the fields studied fluctuated erratically, and oxamyl had little effect on this population pattern. In field 3, both mint growth and Paratylenchus sp. densities were reduced during discing in the fall of 1988. The following spring, Verticillium wilt became a severe problem. A mint rust outbreak made flaming necessary, and

this practice is normally omitted on newly planted or disced fields. A combination of some or all of these factors may have confounded the oxamyl trial results in this field. Paratylenchus sp. densities were lower and hay weights higher in treated plots, but these differences were not significant. The Paratylenchus sp. AUNPC was significantly smaller for treated than for non-treated plots. In field 4, oxamyl apparently had little effect on the Paratylenchus sp. population. The patchy distribution of Paratylenchus sp. and this species' non-responsiveness to oxamyl may explain the lack of significant difference between AUNPCs from different fields or different treatments.

A higher population of C. xenoplax in field 2 in 1989 than in 1988 made no difference in hay or oil production and thus probably had little influence on yield. The effect of this species on peppermint is not known. In the increasing population in field 2, oxamyl appeared effective in keeping the population at low levels, while it appeared ineffective in decreasing the high established population in field 1. C. xenoplax population growth from near zero to moderately high is well documented in nontreated plots in field 2 over the two year study period. The cause of this influx and increase are unknown.

## SUMMARY

Comparisons of AUNPCs appeared to be less a less sensitive measure of differences between treatments, growing seasons, and sampling times than did point sample population analysis. Further evaluation and adjustment of analysis of AUNPCs may facilitate its use as a nematode population assessment tool.

Oxamyl suppressed P. penetrans populations. Suppression was most evident at population peaks, when populations in treated plots remained much lower than those in non-treated plots. Treated populations plateaued in late summer, while non-treated populations increased.

While significantly more hay was produced in treated than in non-treated plots, more oil per unit hay was produced in non-treated plots. Oil/unit field area, however, exhibited no significant differences between treated and non-treated plots.

Paratylenchus sp. was suppressed by oxamyl in a field in which it was nearly a monospecific population. In the other two fields, with high populations of P. penetrans and sometimes C. xenoplax, densities of Paratylenchus sp. were occasionally higher in treated than in non-treated plots.

High populations of C. xenoplax were not suppressed by oxamyl, but low populations in plots treated with oxamyl were prevented from increasing at the same rate as those in non-treated plots.

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